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The Effect of Molybdenum, Tungsten, Copper, Inorganic Sulfur, Phosphorus,and Potassium in Avian Nutrition.

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THE EFFECT OF MOLYBDENUM, TUNGSTEN, COPPER, INORGANIC SULFUR, PHOSPHORUS, AND POTASSIUM IN AVIAN NUTRITION

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ABSTRACT

A STUDY WAS UNDERTAKEN TO DETERMINE THE EFFECT OF CERTAIN MINERAL ELEMENTS IN THE RATION OF GROWING CHICKS, AND IF DESIRABLE RESULTS WERE OBTAINED, TO ATTEMPT TO DETERMINE AN OPTIMUM RATIO BETWEEN THESE ELEMENTS. CERTAIN ENZYMES, WHICH HAVE BEEN REPORTED TO BE ASSOCIATED WITH OR TO CONTAIN SOME OF THESE ELEMENTS WERE STUDIED. IT WAS DESIRABLE TO DETERMINE THE EFFECT OF VARIOUS LEVELS OF SUPPLEMENTATION OF THESE MINERAL ELEMENTS ON THESE ENZYME SYSTEMS. THE MINERAL ELEMENTS INCLUDED MOLYBDENUM, TUNGSTEN, COPPER, INORGANIC SULFUR, PHOSPHORUS, AND POTASSIUM.

THERE WAS FOUND TO BE A CARRY-OVER OF TUNGSTEN FROM DAMS TO CHICKS, AS THE ELEMENT WAS FOUND IN HIGH CONCENTRATIONS IN KIDNEY TISSUES OF CHICKS FROM TUNGSTATE-SUPPLEMENTED DAMS. THIS CONDITION RESULTED IN SIGNIFICANT GROWTH DEPRESSIONS FOR AT LEAST FOUR WEEKS. THIS CONDITION WAS NOT ONLY OVERCOME BUT SIGNIFICANT GAIN INCREASES WERE OBTAINED, COMPARED TO CHICKS FROM NON-SUPPLEMENTED DAMS, WHEN MOLYBDENUM WAS ADDED TO THE RATIONS. THIS WAS FOUND BOTH IN PURIFIED AND PRACTICAL RATIONS AND SUGGESTED A SYNERGISTIC EFFECT BETWEEN MOLYBDENUM AND TUNGSTEN IN THE RATIONS OF GROWING CHICKS. WHEN CERTAIN RATIOS OF MOLYBDENUM AND TUNGSTEN WERE ADDED TO CHICK RATIONS SIGNIFICANT GAIN INCREASES WERE OBTAINED REGARDLESS OF RATION FED OR TREATMENT OF DAMS.

IN RATIONS CONTAINING SUPPLEMENTAL LEVELS OF MOLYBDENUM AND TUNGSTEN, COPPER ADDITIONS WERE FOUND TO SIGNIFICANTLY IMPROVE THE RATIONS. THE SUPPLEMENTAL COPPER CAUSED INCREASES IN GAIN REGARDLESS OF THE DAM

TREATMENT, ALTHOUGH GREATER GAINS WERE FOUND IN CHICKS FROM TUNGSTATE SUPPLEMENTED DAMS. THIS SUGGESTS THAT COPPER MIGHT HAVE OVERCOME A TUNGSTATE TOXICITY IN CHICKS DUE TO THE CARRY-OVER OF TUNGSTEN FROM THE DAMS OR THAT COPPER MAY ACT SYNERGISTICALLY WITH EITHER MOLYBDENUM OR TUNGSTEN.

THE ADDITION OF INORGANIC SULFUR TO RATIONS CONTAINING SUPPLEMENTAL LEVELS OF MOLYBDENUM, TUNGSTEN, AND COPPER WERE FOUND TO BE IMPROVED AS EVIDENCED BY INCREASED GAIN OF CHICKS REGARDLESS OF THE DAM TREATMENT. EVIDENCE WAS PRESENTED WHICH SUGGESTS THAT GROWING CHICKS MAY HAVE A REQUIREMENT FOR INORGANIC SULFATE PER SE.

NO BENEFICIAL EFFECTS WERE FOUND FROM THE ADDITION OF PHOSPHORUS OR POTASSIUM TO RATIONS USED IN THIS STUDY. PHOSPHORUS OR POTASSIUM DID NOT APPEAR TO BE LIMITING FACTORS IN THE RATIONS USED.

THE ADDITION OF 500 PPM TUNGSTEN TO DIETS OF BREEDER HENS WAS FOUND TO CAUSE SIGNIFICANT DEPRESSIONS IN XANTHINE OXIDASE LEVELS OF VARIOUS TISSUES. CHICKS FROM THESE DAMS WERE FOUND TO HAVE SIGNIFICANTLY MORE OF THIS ENZYME THAN DID CHICKS FROM NON-SUPPLEMENTED BREEDER HENS.

THE RATE OF URIC ACID SYNTHESIS DID NOT APPEAR TO BE RELATED TO SUPPLEMENTAL LEVELS OF MOLYBDENUM OR TUNGSTEN IN THE DIET OR TO BE ASSOCIATED WITH GAIN OR FEED CONVERSION RATES OF CHICKS.

DELETERIOUS EFFECTS ON THE RATE OF GROWTH OF CHICKS AT HIGH LEVELS OF SUPPLEMENTATIONS OF MOLYBDENUM, TUNGSTEN, AND COPPER WERE NOTED. THIS WAS ACCOMPANIED BY HIGH ALKALINE PHOSPHATASE ACTIVITY. ALKALINE PHOSPHATASE ACTIVITY APPEARS TO INCREASE DRAMATICALLY DURING PERIODS OF METABOLIC STRESS.

INTRODUCTION

THE DISCOVERY THAT THE ASH OF CERTAIN SOURCES OF UNIDENTIFIED GROWTH FACTORS CONTAINED SUBSTANCES THAT WOULD IMPROVE THE PERFORMANCE OF CHICKENS HAS STIMULATED A RENEWED INTEREST IN THE FIELD OF TRACE MINERAL NUTRITION. THE TERM 'TRACE MINERAL' HAS BEEN USED TO APPLY TO THOSE MINERAL ELEMENTS THAT ARE REQUIRED IN VERY SMALL AMOUNTS IN THE RATION. COMPARATIVELY LITTLE RESEARCH HAS BEEN DONE IN THIS VERY IMPORTANT FIELD. MANY SUGGESTIONS FROM PLANT STUDIES AND ANIMAL STUDIES PROPOSE THAT CERTAIN INTER-RELATIONSHIPS EXISTS BETWEEN SOME OF THESE MINERAL ELEMENTS.

IT HAS BEEN SUGGESTED THAT MOLYBDENUM IS A DIETARY REQUIREMENT FOR CERTAIN ANIMALS. OTHER REPORTS HAVE STATED THAT THIS ELEMENT IS REQUIRED IN CERTAIN ENZYMES, PARTICULARLY IN RATS AND CHICKS. IT HAS ALSO BEEN REPORTED THAT ADDITIONS OF MINUTE AMOUNTS OF MOLYBDENUM IN CHICKS HAS RESULTED IN IMPROVED PERFORMANCE. HOWEVER, HIGHER LEVELS REPORTEDLY RESULT IN TOXIC CONDITIONS. THESE TOXIC CONDITIONS HAVE BEEN ALLEVIATED BY ADDITIONS OF COPPER AND INORGANIC SULFUR IN THE RATIONS OF THESE ANIMALS.

THIS STUDY WAS UNDERTAKEN TO DETERMINE THE EFFECT OF CERTAIN OF THESE MINERAL ELEMENTS IN THE RATION OF GROWING CHICKS, AND IF DESIRABLE RESULTS WERE OBTAINED, TO ATTEMPT TO DETERMINE AN OPTIMUM RATIO BETWEEN SIX OF THESE MINERAL ELEMENTS. CERTAIN ENZYMES, WHICH HAVE BEEN REPORTED TO BE ASSOCIATED WITH OR TO CONTAIN SOME OF THESE ELEMENTS WERE STUDIED. IT WAS DESIRABLE TO DETERMINE THE EFFECT OF

VARIOUS LEVELS OF SUPPLEMENTATION OF THESE MINERAL ELEMENTS ON THESE ENZYME SYSTEMS. THE MINERAL ELEMENTS INCLUDED IN THIS STUDY WERE MOLYBDENUM, TUNGSTEN, COPPER, INORGANIC SULFUR, PHOSPHORUS AND POTASSIUM.

REVIEW OF LITERATURE

Molybdenum

This element was found in minute amounts in a wide range of plant and animal tissues by Ter Meulen (1932) but no special significance was then attached to its presence. It was later shown that molybdenum was required by nitrogen fixing organisms, including the legume symbiont *Rhizobium* species, as well as by *Asperigillus niger* and by higher plants (Arnon and Stout, 1939).

Ferguson *et al* (1938) reported a toxic condition in milking cattle attributed to the action of excess molybdenum. He stated that milk yields fell rapidly, accompanied by a loss in condition of the animals and not infrequently resulted in death. This condition always appeared to be marked by extreme diarrhea. Sheep and other cattle were not as affected as were the milking cattle. There appeared to be no apparent correlation between the total HCl-soluble molybdenum of the soil and that of the herbage.

Teresi *et al* (1942) found that goat's milk was low in molybdenum content (13.5 micrograms per liter). Rats fed goat's milk supplemented with molybdenum as sodium molybdate, did not grow faster than those receiving unsupplemented milk. These workers stated that, if molybdenum were needed by the growing rat, the requirement was approximately 0.5 micrograms per day.

The use of molybdenum in the treatment of chronic copper poisoning of sheep in Australia arose as a result of an earlier observation of

UNUSUALLY HIGH CONCENTRATIONS OF MOLYBDENUM IN LIVER TISSUE OF CATTLE SUFFERING FROM ENZOOTIC HEMATURIA VESICALIS OR "RED WATER", AS REPORTED BY DICK AND BULL (1945). FROM THESE INVESTIGATIONS AND THOSE OF "PEAT SCOURS" IN NEW ZEALAND, CAME A REALIZATION OF THE PROFOUND EFFECT OF MOLYBDENUM ON COPPER METABOLISM IN THE RUMINANT AND OF THE DEPENDENCE OF THIS EFFECT UPON THE INORGANIC SULFATE CONTENT OF THE DIET (UNDERWOOD 1956).

THE TOXICITY OF MOLYBDENUM (AS SODIUM MOLYBDATE) IN A PURIFIED DIET WAS STUDIED IN RATS BY NEILANDS, STRONG, AND ELVEHJEM (1948). A LEVEL OF 500 MILLIGRAMS PER CENT IN THE DIET RESULTED IN DEATH AFTER THE FIRST WEEK, WHEREAS LEVELS OF 50 AND 100 MILLIGRAMS PER CENT GREATLY REDUCED THE GROWTH RATE. BY MEANS OF RADIO-MOLYBDENUM (Mo^{99}) AS A TRACER, THE FATE OF A GIVEN DOSE OF MOLYBDENUM WAS DETERMINED. AT THE END OF TWO DAYS THE KIDNEYS AND BONE CONTAINED SLIGHTLY HIGHER LEVELS OF RADIOACTIVITY PER GRAM THAN DID THE STOMACH AND INTESTINE. ARRINGTON AND DAVIS (1953) REPORTED THAT FEEDING A COMMERCIAL RATION CONTAINING 0.1 PER CENT MOLYBDENUM (AS SODIUM MOLYBDATE) TO DUTCH RABBITS PRODUCED GROSS TOXIC SYMPTOMS. THE TOXIC SYNDROME WAS CHARACTERIZED BY ANOREXIA, LOSS OF WEIGHT, ALOPECIA, DERMATOSIS, ANEMIA, AND DEATH. IN SOME YOUNG RABBITS A DEFORMITY OF THE FRONT LEGS DEVELOPED.

THE DISCOVERY THAT XANTHINE OXIDASE IS A MOLYBDOFLAVOPROTEIN AROSE FROM STUDIES OF DIETARY FACTORS INFLUENCING THE CONCENTRATION OF THIS ENZYME IN THE LIVER OF RATS. LIVER RESIDUES, SOY FLOUR, AND CERTAIN OTHER NATURAL MATERIALS WERE FOUND TO INCREASE THE TISSUE LEVEL OF XANTHINE OXIDASE IN RATS FED SYNTHETIC DIETS. FRACTIONATION OF THESE MATERIALS LED TO THE IDENTIFICATION OF THE "XANTHINE OXIDASE

FACTOR" AS MOLYBDENUM. THIS WAS THE FIRST INDICATION OF AN ESSENTIAL ROLE FOR MOLYBDENUM IN ANIMALS. THESE FINDINGS WERE PUBLISHED FROM TWO DIFFERENT LABORATORIES IN THE SAME YEAR BY DE RENZO ET AL (1953A) AND RICHERT AND WESTERFELD (1953A). IT WAS SUGGESTED (DE RENZO (1953B) THAT PURIFIED DIETS FOR THE RAT SHOULD INCLUDE A SUPPLEMENT OF MOLYBDENUM AT THE RATE OF 40 MILLIGRAMS PER KILOGRAM OF DIET. THIS AMOUNT APPEARED TO PRODUCE A "SATURATION LEVEL" OF XANTHINE OXIDASE IN RAT INTESTINE. INTEREST IN THE BIOLOGICAL SIGNIFICANCE OF MOLYB-DENUM RECEIVED A FURTHER STIMULUS FROM THE DEMONSTRATION THAT THIS ELEMENT IS AN INTEGRAL PART OF THE PROSTHETIC GROUP OF TWO FLAVOPROTEIN ENZYMES; XANTHINE OXIDASE (DE RENZO ET AL 1953A, AND RICHERT AND WESTERFELD 1953A) AND NITROGEN REDUCTASE (NICHOLAS AND MASON 1954).

IN SHEEP AND CATTLE ON LOW MOLYBDENUM DIETS THE AVERAGE CONCEN-TRATION OF MOLYBDENUM IN THE LIVER IS OF THE SAME ORDER OF THAT FOUND FOR RATS, THAT IS, TWO TO FOUR PARTS PER MILLION (HEREAFTER DESIGNATED AS PPM) AS REPORTED BY CUNNINGHAM (1950). SIMILAR LEVELS OF MOLYBDENUM OCCUR IN THE LIVERS OF NEWBORN LAMBS, THUS INDICATING THAT THERE MAY BE NO STORAGE OF THIS ELEMENT DURING PREGNANCY. READY PLACENTAL TRANSFER CAN OCCUR, BECAUSE CONCENTRATIONS THREE TO TEN TIMES THESE "NORMAL" LEVELS HAVE BEEN FOUND IN THE LIVERS OF NEWBORN LAMBS RECEIVING A HIGH-MOLYBDENUM DIET (DICK 1953A) ADULT SHEEP AND COWS HAVE BEEN FOUND TO RETAIN MOLYBDENUM LEVELS OF 25 TO 30 PPM IN THEIR LIVERS AS LONG AS THEY ARE SUPPLIED A MODERATELY HIGH INTAKE OF THIS ELEMENT.

IN CONTRAST TO IRON AND COPPER, MOLYBDENUM IS READILY AND APPAR-ENTLY RAPIDLY ABSORBED FROM THE INTESTINAL TRACT. THIS APPLIES ESPECIALLY TO HEXAVALENT WATER SOLUBLE FORMS OF MOLYBDENUM SUCH AS

SODIUM OR AMMONIUM MOLYBDATE, WHICH HAVE BEEN USED IN MOST EXPERIMENTAL STUDIES, AND TO MOLYBDENUM OF HERBAGE, A HIGH PROPORTION OF WHICH, AT LEAST IN HIGH MOLYBDENUM PASTURES, IS WATER SOLUBLE (UNDERWOOD 1956). THERE IS SOME EVIDENCE THAT EVEN SUCH INSOLUBLE COMPOUNDS AS MOLYBDENUM TRIOXIDE AND CALCIUM MOLYBDATE ARE ALSO READILY ABSORBED IN RABBITS AND GUINEA PIGS WHEN THESE COMPOUNDS ARE FED. THIS DOES NOT APPEAR TO BE TRUE FOR MOLYBDENITE (MoS_2) (FAIRHALL ET AL 1945).

MOST OF THE AVAILABLE EVIDENCE INDICATES THAT MOLYBDENUM IS EXCRETED MAINLY IN THE URINE. STUDIES WITH GUINEA PIGS AND RABBITS ADMINISTERED VARIOUS FORMS OF STABLE MOLYBDENUM OVER A WIDE RANGE OF DOSES SUPPORT THIS HYPOTHESIS (UNDERWOOD 1956). ADDITIONAL SUPPORT COMES FROM AN INVESTIGATION IN WHICH RADIOMOLYBDENUM (Mo^{99}) AS Na_2MoO_4 WAS ADMINISTERED TO A STEER. WHEN GIVEN ORALLY, ABOUT 34 PER CENT OF THE MOLYBDENUM APPEARED IN THE FECES WITHIN 14 DAYS AND 45 PER CENT WAS EXCRETED IN THE URINE. FOLLOWING INTRAVENOUS INJECTION, ABOUT 11 PER CENT OF THE DOSE WAS FOUND IN THE FECES AND 37 PER CENT IN THE URINE AFTER SIX DAYS (COMAR 1950).

ANIMAL SPECIES APPEAR TO VARY IN THEIR TOLERANCE TO HIGH INTAKES OF MOLYBDENUM AND IN THE TOXIC SYMPTOMS THAT ARE DISPLAYED. CATTLE ARE THE LEAST TOLERANT, FOLLOWED BY SHEEP. HORSES AND PIGS APPEAR TO BE THE MOST TOLERANT, WITH RATS, RABBITS, AND GUINEA PIGS SEEMINGLY OCCUPYING AN INTERMEDIATE POSITION (UNDERWOOD 1956). THE MINIMUM TOXIC DIETARY LEVELS OF MOLYBDENUM CANNOT BE FIXED FOR ANY OF THESE SPECIES ON THE BASIS OF PRESENT EVIDENCE BECAUSE OF THE NUMBER OF INFLUENCING FACTORS - SOME KNOWN, SOME STILL UNKNOWN. ACCORDING TO FAIRHALL ET AL (1945) THE TOLERANCE TO MOLYBDENUM IS AFFECTED BY THE CHEMICAL FORM IN WHICH THE MOLYBDENUM IS ADMINISTERED, THE COPPER

CONTAINED IN THE ORGANISM, COPPER INTAKE OF THE ANIMAL, AND THE INORGANIC SULFATE IN THE DIET (ESPECIALLY FOR SHEEP). THE LEVEL OF INTAKE OF OTHER METALS INCLUCING ZINC AND LEAD AS WELL AS THE INTAKE OF METHIONINE MAY ALSO HAVE AN EFFECT. SIMILAR EVIDENCE HAS ALSO BEEN PRESENTED BY GRAY AND ELLIS (1950).

THAT THE ADDITION OF 0.126 PPM MOLYBDENUM, AS SODIUM MOLYBDATE, TO A PURIFIED DIET INCREASED THE GROWTH OF MOLYBDENUM DEPLETED CHICKS 10 TO 18 PER CENT, WAS REPORTED BY REID AND ASSOCIATES (1956A). IN THIS DIET, MINERAL MIXTURES WHICH SIMULATED THE ASH OF DISTILLERS DRIED SOLUBLES WERE FOUND TO PRODUCE GROWTH RESPONSES WITH BOTH CHICKS AND POULTS. THE OMISSION OF MOLYBDENUM FROM SUCH A MIXTURE DID NOT APPRECIABLY AFFECT THE GROWTH PROMOTING PROPERTY. IT SHOULD BE NOTED THAT THESE WORKERS ADMITTED THAT THE CHEMICALS USED IN THE RECONSTITUTED ASH MAY NOT HAVE BEEN FREE OF MOLYBDENUM. ALTHOUGH THE BASAL DIET CONTAINED 1.0 TO 1.1 PPM MOLYBDENUM, THESE WORKERS ASSUMED THAT THE MOLYBDENUM WAS FIRMLY BOUND IN THE SOYFLOUR OF THE RATION AND MAY NOT HAVE BEEN AVAILABLE TO THE CHICKS. FURTHER WORK REPORTED BY REID AND CO-WORKERS (1956B) SUPPORTED THE CHICK GROWTH RESPONSE DATA AND ALSO FOUND THAT MOLYBDENUM ADDED AT SUPPLEMENTAL LEVELS OF LESS THAN 0.5 PPM IN A PURIFIED DIETS PRODUCED A GROWTH RESPONSE IN TURKEY POULTS.

MOTZOK ET AL (1957) REPORTED EXPERIMENTS WITH PRACTICAL RATIONS IN WHICH MOLYBDENUM WAS ADDED (AS SODIUM MOLYBDATE) AT SUPPLEMENTAL LEVELS OF 0, 50, 100, 200, AND 500 PPM. AT EIGHT WEEKS OF AGE THERE WAS A MARKED DEPRESSION IN GROWTH OF PULLETS AND COCKERELS OF THREE BREEDS OF CHICKS FED THE DIET CONTAINING 500 PPM MOLYBDENUM. THE BASAL RATION REPORTEDLY CONTAINED SEVEN PPM MOLYBDENUM AND NO GROWTH

RESPONSE WAS NOTED WHEN SUPPLEMENTED WITH THE OTHER LEVELS OF MOLYBDENUM. HOWEVER, IT WAS FOUND THAT THE MOLYBDENUM CONTENT OF THE BLOOD AND LIVER WAS INCREASED WITH ALL LEVELS OF MOLYBDENUM WHEN ADDED TO THE DIET, THE GREATEST INCREASE BEING 300-FOLD IN THE BLOOD AND SEVEN-FOLD IN THE LIVER AT THE HIGHEST LEVEL OF MOLYBDENUM FED. IT WAS ALSO FOUND THAT THE MOLYBDENUM CONTENT OF EGGS WAS INCREASED WHEN MOLYBDENUM WAS ADDED TO THE RATION.

THE ADDITION OF MOLYBDENUM (AS SODIUM MOLYBDATE) TO A PURIFIED AND TO A PRACTICAL CHICK DIET AT LEVELS TO GIVE LESS THAN ONE PPM PROMOTED GROWTH AND INCREASED XANTHINE DEHYDROGENASE ACTIVITY OF THE LIVER AND INTESTINAL TISSUES OF CHICKS AT FOUR AND TEN WEEKS OF AGE, ACCORDING TO KURNICK AND ASSOCIATES (1957). MOLYBDENUM STUDIES WITH RATS, CHICKS, AND TURKEYS FED A PURIFIED CASEIN DIET CONTAINING 0.1 PPM MOLYBDENUM HAVE INDICATED THAT THE AVAILABILITY OF THE ELEMENT WAS INFLUENCED NOT ONLY BY THE GENUS OF THE ANIMAL BUT ALSO BY THE DIRECTION AND MAGNITUDE OF THE GENUS DIFFERENCE. THIS IS IN AGREEMENT WITH WORK BY REID AND ASSOCIATES (1957) WHO REPORTED THAT BOTH LIVER AND INTESTINAL XANTHINE DEHYDROGENASE LEVELS OF CHICKS FED MOLYBDENUM SUPPLEMENTED RATIONS WERE SIGNIFICANTLY GREATER THAN THOSE OF BIRDS ON A CONTROL RATION. DIFFERENCES AS LARGE AS TWO PER CENT IN BONE ASH WERE OBSERVED BETWEEN BIRDS ON RATIONS CONTAINING 0.0254 PPM ADDED MOLYBDENUM AND THOSE ON THE CONTROL RATION. HOWEVER, IT WAS FOUND THAT THESE DIFFERENCES IN BONE ASH WERE NOT STATISTICALLY SIGNIFICANT.

IN A REVIEW OF MOLYBDENUM FINDINGS, UNDERWOOD (1957) REPORTED THAT MOLYBDENUM IS KNOWN TO BE A COMPONENT OF THE ENZYME XANTHINE OXIDASE. HE POINTED OUT THAT THIS ENZYME LEVEL HAS BEEN REPORTED TO VARY IN TISSUES OF ANIMALS FED DIFFERENT LEVELS OF MOLYBDENUM. IT HAS

BEEN REPORTED THAT THIS ELEMENT STIMULATES GROWTH. HOWEVER, THIS FINDING HAS NOT BEEN UNIFORMLY SUCCESSFUL IN OTHER LABORATORIES EXCEPT WHEN A MOLYBDENUM ANTAGONIST, SUCH AS SODIUM TUNGSTATE, HAS BEEN ADDED TO THE DIET. IT SEEMS REASONABLE, NEVERTHELESS, TO ACCEPT MOLYBDENUM TENTATIVELY AS AN ESSENTIAL TRACE ELEMENT WHILE FURTHER RESEARCH IS CARRIED OUT IN WHICH MORE ACUTELY DEFICIENT DIETS ARE USED AND MOLYBDENUM LEVELS IN THE TISSUES ARE MEASURED.THERE IS ALSO THE DIFFICULT PROBLEM OF THE AVAILABILITY OF MOLYBDENUM IN DIFFERENT FORMS AND FROM DIFFERENT DIETARY SOURCES. THESE VIEWS ARE NOT WHOLLY SHARED BY McELROY AND NASON (1956), WHO SUMMIZE THAT IN RECENT YEARS IT HAS BECOME FASHIONABLE TO ASSUME THAT A METAL IS ESSENTIAL IF IT IS FOUND TO BE REQUIRED SPECIFICALLY FOR THE FUNCTION OF ANY ENZYME. THIS IS A REASONABLE ASSUMPTION ONLY IF THE ENZYME IS A NECESSARY ONE. THESE WORKERS QUESTION THAT XANTHINE OXIDASE IS ESSENTIAL FOR GROWTH AND REPRODUCTION. HOWEVER, IF IT IS ESSENTIAL AND MOLYBDENUM IS REQUIRED FOR ITS SYNTHESIS OR FUNCTION, THEN KNOWLEDGE CONCERNING THE INDUCED FORMATION OF ENZYMES SUGGESTS THAT SOME OF THESE CATALYSTS CAN BE ELIMINATED UNDER CERTAIN NUTRITIONAL CONDITIONS WITHOUT AFFECTING THE VIABILITY OF THE ORGANISM. THUS WHAT MAY BE AN ESSENTIAL ELEMENT WITH ONE DIET MAY NOT BE FOR A SECOND DIET.

REMY AND WESTERFELD (1951) REPORTED THE EFFECT OF SEVERAL DIET FACTORS ON THE XANTHINE DEHYDROGENASE CONTENT OF CHICKEN TISSUES. THEY FOUND THAT FOLIC ACID INHIBITED PURIFIED CHICKEN LIVER XANTHINE DEHYDROGENASE <u>IN VITRO</u>, PRESUMABLY BECAUSE OF THE PRESENCE OF 6-PTERIDYALDEHYDE IN THE FOLIC ACID. HOWEVER, DIETARY FOLIC ACID DID NOT DECREASE THE LEVEL OF THE ENZYME IN THE KIDNEY, PANCREAS OR INTESTINE, AND WAS ACTUALLY REQUIRED FOR ITS DEPOSITION IN THE

INTESTINE WHEN THE PROTEIN INTAKE WAS HIGH. DIETARY LIVER RESIDUE HAD LITTLE EFFECT ON THIS ENZYME CONCENTRATION IN ANY CHICKEN TISSUE.

AMMONIUM MOLYBDATE IN CONCENTRATIONS FROM 0.005 TO 0.05 MOLAR WAS ADDED TO 5.4 PER CENT GLUCOSE SOLUTION TO TEST THE EFFECT OF MOLYBDATE ON INTESTINAL ABSORPTION OF GLUCOSE IN THE RAT (PONY, 1953). WITH 0.005 MOLAR MOLYBDENUM THERE WAS NO EFFECT ON GLUCOSE ABSORPTION, BUT INHIBITION WAS EVIDENT WITH 0.025 MOLAR SOLUTIONS. A CONCENTRATION OF 0.05 MOLAR CAUSED INHIBITION BY 20 TO 50 PER CENT IN ONE ABSORPTION TEST AND 30 TO 50 PER CENT IN A SECOND. IT WAS SUGGESTED THE INHIBITING EFFECT OF MOLYBDENUM WAS PRESUMABLY DUE TO A LOCAL EFFECT ON THE EPITHELIAL CELLS.

ACCORDING TO DERENZO AND ASSOCIATES (1953B) DIETS CONTAINING 0.02 MILLIGRAMS MOLYBDENUM PER GRAM WERE SUFFICIENT TO PRODUCE A "SATURATION LEVEL" OF XANTHINE OXIDASE IN RATS. ASSUMING A DAILY FOOD INTAKE OF 10 TO 15 GRAMS, IT WOULD APPEAR THAT THE MAXIMUM DAILY REQUIREMENT TO PRODUCE THIS EFFECT WOULD BE ON THE ORDER OF 0.2 TO 0.3 MILLIGRAM MOLYBDENUM PER RAT. THE TOTAL ACTIVITY OF LIVER RESIDUE, CALCULATED TO CONTAIN APPROXIMATELY 1.2 MILLIGRAMS MOLYBDENUM PER GRAM BY SPECTROGRAPHIC ANALYSIS, CAN EASILY BE ACCOUNTED FOR ON THE BASIS OF ITS MOLYBDENUM CONTENT. IN LATER WORK BY DERENZO, HEYTLER, AND KALEITA (1954) WEANLING RATS WERE MADE DEFICIENT OF MOLYBDENUM. THEY WERE THEN GIVEN SINGLE SUBCUTANEOUS INJECTIONS OF 40 MICROGRAMS MOLYBDENUM AND SACRIFICED BY DECAPITATION AT 1.4 AND 8.0 HOURS AFTER INJECTION. THE INTESTINES WERE REMOVED AND XANTHINE OXIDASE DETERMINED. THEY FOUND THAT RAPID FORMATION OF XANTHINE OXIDASE WAS EVIDENT AFTER THE SUBCUTANEOUS INJECTIONS OF MOLYBDENUM. IN CONTRAST, THE INJECTION OF VITAMIN B_{12} INTO VITAMIN B_{12}

DEFICIENT RATS WAS REPORTED TO INCREASE THE LEVEL OF LIVER XANTHINE OXIDASE ONLY AFTER A PERIOD OF EIGHT DAYS.

XANTHINE OXIDASE REQUIRES MOLYBDENUM FOR ACTIVITY WITH CYTOCHROME c, BUT NOT WITH DYES AND OXYGEN, THUS CONFORMING TO THE PATTERNS SET BY OTHER METALLO-FLAVOPROTEINS (MACKLER AND ASSOCIATES, 1954). MOLYBDENUM WAS REMOVED FROM THE ENZYME BY DIALYSIS AGAINST 0.01 MOLAR AMMONIA, AND ACTIVITY WAS RESTORED BY THE ADDITION OF MOLYBDIC OXIDE. THIS ACTIVITY IS DEPENDENT ON THE PRESENCE OF INORGANIC PHOSPHATE AND IS COMPETITIVELY INHIBITED BY PYROPHOSPHATE. ENZYMATIC ACTIVITY WITH DIPHOSPHOPYRIDINE NUCLEOTIDE RESIDES IN THE SAME PROTEIN WITH WHICH XANTHINE OXIDASE ACTIVITY IS ASSOCIATED, BUT MAY OCCUR AT A SITE DIFFERENT FROM THAT INVOLVED WITH OTHER SUBSTRATES, SUCH AS ALDEHYDES OR PURINES. IN ADDITION TO FLAVIN ADENINE DINUCLEOTIDE AND MOLYBDENUM, THE ENZYME APPEARS TO CONTAIN A SECOND CHROMOPHORIC GROUP. THE PARTIAL REDUCTION OF FLAVIN BY HYPOXANTHINE IS PROBABLY DUE TO AN OXIDATION-REDUCTION EQUILIBRIUM AND NOT TO THE PRESENCE OF INACTIVE FLAVIN ADENINE DINUCLEOTIDE.

NICHOLAS AND NASON (1954) STATED THAT MOLYBDENUM HAS BEEN IDENTIFIED AS THE METAL CONSTITUENT OF NITRATE REDUCTASE. THEIR DATA SHOWED THAT THE CONTENT OF THE TRACE METAL INCREASED WITH THE SPECIFIC ACTIVITY OF THE ENZYME. AMMONIUM SULFATE FRACTIONS OF NITRATE REDUCTASE CAN BE DIALYZED IN MEMBRANES WHICH HAVE BEEN PRETREATED IN GLUTATHIONE-PHOSPHATE SOLUTIONS. ACTIVATION OF THE ENZYME, DIALYZED AGAINST CYANIDE AND SUBSEQUENTLY AGAINST PHOSPHATE AND GLUTATHIONE TO REMOVE EXCESS CYANIDE AND METALLOCYANIDE COMPLEXES, WAS ACCOMPLISHED BY MOLYBDENUM TRIOXIDE OR SODIUM MOLYBDATE. OTHER MICRO-NUTRIENTS INCLUDING IRON, COPPER, ZINC, MANGANESE, COBALT,

NICKEL, TUNGSTEN, VANADIUM, URANIUM, AND BORON, RESPECTIVELY, WERE WITHOUT EFFECT ON THE DIALYZED ENZYME.

THE ADDITION OF SODIUM TUNGSTATE (94 MILLIGRAMS PER KILOGRAM) TO A PURIFIED CASEIN DIET VIRTUALLY ELIMINATED THE XANTHINE OXIDASE FROM RAT AND CHICKEN TISSUES WHEN THE ENZYME ACTIVITY WAS MANOMETRICALLY MEASURED. IN CHICKS, HALF OF THE PURINES EXCRETED CONSISTED OF XANTHINE AND HYPOXANTHINE, BUT URIC ACID AND ALLANTOIN EXCRETIONS IN RATS WERE UNAFFECTED ACCORDING TO RICHERT AND WESTERFELD (1957A) THIS COULD MEAN THAT (1) SMALL AMOUNTS OF XANTHINE OXIDASE ESCAPED DETECTION BY THE MANOMETRIC PROCEDURE OR (2) URIC ACID WAS FORMED BY ANOTHER PATHWAY IN RATS. LATER (RICHERT, BLOOM AND WESTERFELD 1957) IT WAS FOUND THAT HOMOGENATES OF LIVERS FROM RATS DEPLETED OF MOLYBDENUM AND XANTHINE OXIDASE BY TUNGSTATE FEEDING DID NOT SHOW AN INCREASED OXYGEN UPTAKE WITH EITHER FREE PURINES OR THEIR NUCLEOSIDES OR NUCLEOTIDES AS SUBSTRATES. THE ADDITION OF DIPHOSPHOPYRIDINE NUCLEOTIDE AND METHYLENE BLUE DID NOT RESTORE ANY OF THESE OXIDIZING CAPACITIES, BUT MILK XANTHINE OXIDASE DID. SMALL AMOUNTS OF XANTHINE OXIDASE WERE PRESENT IN SUCH LIVERS, BUT ESCAPED DETECTION BY THE MANOMETRIC PROCEDURE BECAUSE ENDOGENOUS SUBSTRATES WERE BEING OXIDIZED MAXIMALLY IN THE BLANK VESSEL, AND ADDITIONAL SUBSTRATE COULD NOT INCREASE THIS RATE. THE PRESENCE OF XANTHINE OXIDASE WAS DEMONSTRATED BY MEASURING ALLANTOIN FORMATION FROM THE VARIOUS SUBSTRATES, AND ENOUGH XANTHINE OXIDASE WAS FOUND TO ACCOUNT FOR THE FORMATION OF THE URIC ACID AND ALLANTOIN NORMALLY EXCRETED BY THE RAT. NO EVIDENCE COULD BE OBTAINED THAT URIC ACID ACID WAS FORMED IN RAT LIVER BY ANY MECHANISM OTHER THAN XANTHINE OXIDASE.

ACCORDING TO MAHLER ET AL (1954) ALDEHYDE OXIDASE IS A MOLYBDOFLAVO PROTEIN. THE METAL IS REQUIRED FOR INTERACTION WITH CYTOCHROME

C, BUT NOT WITH DYES AND OXYGEN. MOLYBDENUM CAN BE REMOVED BY DIALYSIS OF THE ENZYME AGAINST 0.01 MOLAR AMMONIA. FULL ACTIVITY IS RESTORED BY THE ADDITION OF MOLYBDIC TRIOXIDE, BUT NO OTHER METAL ION TESTED WAS ACTIVE. THE MOLYBDENUM-CATALYZED REACTION REQUIRES THE PRESENCE OF INORGANIC PHOSPHATE. SILICATE, AND TO A LESSER EXTENT, ARSENATE CAN TAKE THE PLACE OF PHOSPHATE WHILE PYRO-PHOSPHATE COMPETITIVELY INHIBITS THE ADDITION OF PHOSPHATE. RICHERT AND WESTERFELD (1957a) REPORTED THAT BOTH XANTHINE OXIDASE AND ALDEHYDE OXIDASE ARE MOLYBDENUM CONTAINING ENZYMES, AND BOTH ARE CAPABLE OF OXIDIZING ALDEHYDES. ANOTHER ALDEHYDE-OXIDIZING ENZYME UTILIZING DIPHOSPHOPYRIDINE NUCLEOTIDE AS A COFACTOR HAS BEEN PARTIALLY PURIFIED FROM LIVER, AND THE OXIDATION OF ACETALDEHYDE BY CRUDE LIVER EXTRACTS REQUIRES DIPHOSPHOPYRIDINE NUCLEOTIDE. A STUDY WAS DESIGNED TO SEE WHETHER THE DPN ENZYME WAS ALSO MOLYBDENUM-DEPENDENT AND WHICH OF THESE SYSTEMS WAS PRIMARILY RESPONSIBLE FOR ACETALDEHYDE OXIDATION IN DIALYZED LIVER HOMOGENATES. RATS WERE FED A DIET CONTAINING TUNGSTATE TO DEPLETE THE TISSUES OF MOLYBDENUM-CONTAINING ENZYMES. THE RATE OF ACETALDEHYDE OXIDATION IN LIVER HOMOGENATES WAS MEASURED IN THE PRESENCE AND ABSENCE OF DPN. IT WAS FOUND THAT, WHILE THE CAPACITY TO OXIDIZE ACETALDEHYDE IN THE PRESENCE OF DPN WAS NOT AFFECTED, FEEDING A PROTEIN DEFICIENT DIET CAUSED THE VIRTUAL ELIMINATION OF ALL THE ALDEHYDE OXIDIZING ENZYMES STUDIED. THE DPN-LINKED ACETALDEHYDE OXIDIZING ENZYME OF RAT LIVER WAS FOUND TO BE INDEPENDENT OF DIETARY MOLYBDENUM.

Copper

For many years the presence of copper in biological materials was assumed to be merely accidental. It was not until the 1920's that the universal distribution of copper in plant and animal tissues became well recognized and a physiological function for this element was definitely established.

Definite evidence that copper is an essential dietary component with a specific function within the animal body arose from studies which were initiated at Wisconsin in 1924, on hemoglobin regeneration in rats suffering from milk anemia. After a series of experiments, Hart and associates (1928) announced that copper in addition to iron was essential for the formation of hemoglobin in the rat. This discovery was almost immediately confirmed by other laboratories and established the fact that copper is essential for hematopoesis in a wide range of animal species as recorded by Elvehjem and Hart (1929) and Elvehjem (1935).

That the copper content of a rat at birth was not raised by subjecting the mother to a high copper intake was reported by Lindow et al (1929). There was no indication of increased placental transmission, nor was there increased transmission of copper through the milk. Feeding of additional copper led to marked increases in the absolute copper content and the percentage of this element present in the bodies of rats of various ages fed different rations. The absolute amount of copper in the body of a rat on an adequate ration gradually increased from 0.0198 milligrams at birth to 0.4422 milligrams at 210 to 240 days. On a percentage basis there was a constant decrease from birth up to 85 days and a slight increase at 210 to 240 days. This increase was most

STRIKING IN THE MORE ADULT ANIMALS WHICH AT 75 TO 85 DAYS OF AGE CONTAINED TWO, AND AT 210 TO 240 DAYS, THREE TIMES AS MUCH COPPER AS THE ANIMALS FED THE STOCK RATION ALONE.

CUNNINGHAM (1931) REPORTED THAT THE COPPER CONTENT OF THE SKIN AND LIVERS OF RATS WERE INCREASED A RELATIVELY SMALL DEGREE COMPARED WITH LEVELS OF ADDITIONAL COPPER FED IN THE DIET. IN CONTRAST TO THIS EXPERIMENTOR, LINDOW AND ASSOCIATES (1929) FOUND THAT FEEDING ADDITIONAL COPPER TO THE MOTHER CAUSED INCREASED STORAGE OF COPPER IN THE NEWLY BORN RAT. THEY ALSO REPORTED THAT SUPPLEMENTAL COPPER HAD LITTLE OR NO EFFECT ON THE STORAGE OF IRON IN THE FETAL RAT OR IN THE HEN'S EGG.

ELVEHJEM AND ASSOCIATES (1930) FOUND THE AVERAGE COPPER CONTENT OF EGG YOLK TO BE 0.0076 PER CENT AND OF EGG ALBUMIN TO BE 0.0056 PER CENT. THE AMOUNT OF COPPER IN THE YOLK AND ALBUMIN WAS NOT INCREASED BY FEEDING THE HEN 50 MILLIGRAMS OF IRON OR 50 MILLIGRAMS OF IRON PLUS 0.5 MILLIGRAM COPPER DAILY.

FOLLOWING THE DEMONSTRATION OF THE IMPORTANCE OF COPPER IN HEMATOPOESIS, THERE WAS A RAPID INCREASE IN KNOWLEDGE OF OTHER BIOLOGICAL FUNCTIONS OF COPPER. A NUMBER OF COPPER PROTEIN COMPOUNDS WERE ISOLATED FROM BOTH PLANT AND ANIMAL SOURCES, SEVERAL OF WHICH WERE SHOWN TO BE OXIDASES [TYROSINASE (POLYPHENOL OXIDASE), LACCASE, AND ASCORBIC ACID OXIDASE] ACCORDING TO KIELIN AND MANN (1938). MORE RECENTLY BUTYRL CO-A DEHYDROGENASE HAS BEEN IDENTIFIED AS A CUPROFLAVOPROTEIN IN WHICH COPPER OCCURS AS A PART OF THE PROSTHETIC GROUP (MAHLER 1953). THE ACTIVITIES OF THESE ENZYMES ARE DEPENDENT UPON THE COPPER THEY CONTAIN; BUT AS YET THERE APPEARS TO BE LITTLE KNOWN ABOUT HOW COPPER IS BOUND, OR HOW IT FUNCTIONS WITHIN THE ENZYME.

ALSO IT IS NOT CLEAR WHAT QUANTITATIVE SIGNIFICANCE CAN BE ATTACHED TO THESE COPPER-CONTAINING ENZYMES IN THE OXIDATIVE PROCESSES OF THE INTACT CELL.

IN WORK DONE SOMEWHAT EARLIER, SMITH AND DEDLICOTT (1944) FOUND THAT A DEFICIENCY OF EITHER IRON OR COPPER PRODUCES AN ANEMIA IN THE RAT WHICH WAS MORPHOLOGICALLY CLASSIFIED AS MICROCYTIC AND HYPOCHROMIC. THE FEEDING OF PURE IRON TO MILK ANEMIC RATS LEAD TO A SINGIFICANT INCREASE IN THE MEAN CELL VOLUME, WHICH STILL REMAINED LESS THAN NORMAL. THE FEEDING OF COPPER TO THESE MILK ANEMIC RATS PRODUCED A RISE IN THE ERYTHROCYTE COUNT WHICH WAS NOT ACCOMPLISHED BY AN INCREASE IN HEMOGLOBIN.

CERTAIN NATURALLY-OCCURRING DISEASES OF GRAZING SHEEP AND CATTLE WERE FOUND TO BE DUE TO A DEFICIENCY OF COPPER AND COBALT, OR WHICH RESPONDED TO COPPER THERAPY. ONE OF THE FIRST REPORTS SUGGESTED THAT A DEFICIENCY OF COPPER OCCURS IN LIVESTOCK WAS THAT OF NEAL AND ASSOCIATES (1931) AS A RESULT OF THEIR STUDIES OF "SALT-SICK" COWS IN FLORIDA.

REPORTS HAVE BEEN PUBLISHED ON THE COPPER CONTENT OF THE PRINCIPLE TISSUES AND ORGANS OF VARIOUS ANIMALS, INCLUDING THOSE OF THE RAT, RABBIT, CAT, DOG, PIG, GUINEA PIG, SHEEP, HORSE, COW, DOMESTIC FOWL, AND MAN. VARYING CONCENTRATIONS HAVE BEEN FOUND IN EVERY ORGAN EXAMINED AND THERE SEEMS TO BE REASON TO ASSUME THAT COPPER OCCURS IN ALMOST, IF NOT ALL, BODY CELLS. INIDIVIDUAL VARIABILITY APPEARS TO BE TOO LARGE TO ALLOW DEFINITE VALUES TO BE ASSIGNED TO SPECIFIC TISSUES OR ORGANS. DATA OF THIS NATURE HAVE BEEN REPORTED BY BRUCKMANN AND ZONDEK (1940), CUNNINGHAM (1931), LORENZEN AND SMITH (1947), AND MARSTON (1952). THE ENDOCRIN GLANDS <u>I.E.</u> PITUITARY,

THYROID, AND THYMUS, ARE REPORTED EXAMPLES OF ORGANS WITH LOW COPPER CONCENTRATIONS WHILE THE LIVER, KIDNEYS, HEART, HAIR, AND BRAIN ARE AMONG THOSE OF HIGHER CONCENTRATIONS OF COPPER.

FROM THE AVAILABLE EVIDENCE IT APPEARS NEITHER A DEFICIENCY NOR AN EXCESS OF DIETARY COPPER GREATLY INFLUENCES THE COPPER CONTENT OF CERTAIN OF THESE TISSUES AND ORGANS, AND ESPECIALLY THE ENDOCRINE GLANDS, MUSCLES, HEART AND SKIN. IT HAS BEEN REPORTED THAT THE COPPER CONTENT OF THE LIVER, KIDNEY, SPLEEN, AND LUNGS CAN BE GREATLY INCREASED BY HIGH COPPER INTAKE, AND THAT OF THE LIVER, KIDNEY, SPLEEN, HAIR, AND BLOOD CONSIDERABLY REDUCED UNDER CONDITIONS OF COPPER DEFICIENCY (LINDOW AND CO-WORKERS 1929). THESE WORKERS FURTHER STATED THAT THE ADDITION OF FIVE MILLIGRAMS OF COPPER PER DAY TO A NORMAL STOCK RATION RAISED THE COPPER CONCENTRATION OF ADULT RAT LIVERS FROM 11 TO 213 PPM (DRY BASIS), THE KIDNEY FROM 12 TO 17 PPM AND THE SPLEEN FROM THREE TO 17 PPM WITH VERY LITTLE CHANGE IN COPPER LEVELS IN THE HEART, BRAIN, SKIN AND MUSCLES.

SEEMINGLY, VERY LITTLE IF ANYTHING IS KNOWN OF THE MECHANISM OF ABSORPTION OF COPPER, AND THE FACTORS WHICH INFLUENCE ITS ABSORPTION ARE VERY IMPREFECTLY UNDERSTOOD. TOMPSETT (1940) STATED THAT ABSORPTION OF COPPER PROBABLY OCCURS IN MAN IN THE UPPER PART OF THE SMALL INTESTINE WHERE THE CONTENTS STILL HAVE A PRONOUNCED ACID REACTION. HE STATED THAT THE REACTION OF THE INTESTINAL CONTENTS MUST EXERT A MARKED INFLUENCE ON COPPER ABSORPTION AND THIS, IN TURN, MUST DEPEND ON A NUMBER OF FACTORS SUCH AS THE GASTRIC ACIDITY, THE BASE (I.E. CALDIUM) CONTENT OF THE DIET AND THE CHARACTER OF THE INTESTINAL SECRETIONS.

IT IS ALSO POSSIBLE THAT METABOLIC CHANGES IN PLANTS CAUSE COPPER TO ENTER COMBINATIONS FROM WHICH IT CANNOT BE READILY RELEASED AND ASSIMILATED BY ANIMALS. A HIGHLY INTERESTING INVESTIGATION IN THIS ASPECT OF NUTRITIONAL PHYSIOLOGY OF COPPER WAS MADE BY MILLS (1954). HE POINTED OUT THAT THE GREATER PORTION OF COPPER IN HERBAGE EXISTS IN A BOUND FORM AND THAT MUCH OF THE COPPER IS NOT EXTRACTABLE BY ORGANIC SOLVENTS OR BY DILUTE AQUEOUS SOLUTIONS OF ORGANIC CHELATING AGENTS. HE FURTHER STATED THAT SEASONAL VARIATIONS IN THE SOLUBILITY OF HERBAGE COPPER EXIST AND THAT LESS WATER SOLUBLE COPPER IS PRESENT IN PASTURES IN WHICH "SWAYBACK" IN LAMBS OCCURS THAN IN NORMAL HERBAGE. EVIDENCE WAS FURTHER PRESENTED THAT A STABLE, WATER-SOLUBLE COPPER COMPLEX PRESENT IN HERBAGE IS MORE READILY UTILIZED BY THE COPPER-DEFICIENT RAT THAN CUPRIC ION.

A STUDY WAS MADE OF THE COPPER TOLERANCE OF YOUNG CHICKENS BY MAYO ET AL, (1956). GROWTH RATE, MORTALITY, AND OCCURRENCE OF MUSCULAR DYSTROPHY SERVED AS INDICATORS OF COPPER TOLERANCE. A PURIFIED DIET, CONSISTING PRIMARILY OF CERELOSE, CASEIN, GELATIN, AND A CORN-SOYBEAN MEAL DIET WERE BOTH USED IN THE STUDY. IN ONE TRIAL THE CORN-SOYBEAN MEAL DIET WITH 324 PPM COPPER CAUSED MUSCULAR DYSTROPHY AND SIGNIFICANT INHIBITION OF GROWTH AT FOUR WEEKS OF AGE. IN ANOTHER TRIAL A LEVEL OF 520 PPM COPPER DECREASED GROWTH AT FOUR WEEKS OF AGE. WHEN COPPER WAS INCLUDED AS COPPER-BOUND-CASEIN INSTEAD OF COPPER SULFATE, A SIGNIFICANT GROWTH INCREASE RESULTED, BUT THE INCIDENCE OF MUSCULAR DYSTROPHY REMAINED THE SAME. NO RELATIONSHIP BETWEEN THE INCIDENCE OF MUSCULAR DYSTROPHY AND VITAMIN E LEVEL OF THE RATION WAS ESTABLISHED. A MARKED INCREASE IN MORTALITY OCCURRED IN THE CHICKS WHEN EITHER A PRACTICAL OR PURIFIED DIET CONTAINED 1270 PPM COPPER.

Phosphorus

There are not many reports which furnish a basis for differentiation of the phytin phosphorus of cereals and the more available forms of phosphorus combined in bone meal, mineral phosphates, animal protein sources, fats and proteins. Since 1939 it has been clear that phytin phosphorus is, at best, poorly available to the chick and for practical purposes should hardly be considered in estimating the phosphorus requirement (Almquist, 1954). This may be particularly true in reference to modern high-efficiency diets which are usually low in potent sources of phytase.

McGinnis and associates (1944) reported that chick rations containing 58 per cent phosphorus from vegetable sources could not support maximal calcification with any level of vitamin D up to as much as 320 A.O.A.C. chick units per 100 grams. The data on bone ash values showed a linear relationship to the logarithm of vitamin D intake. Such data suggest that there was a constant suboptimal level of available phosphorus the utilization of which was increased with vitamin D. When all phosphorus was in inorganic form as little as twenty units vitamin D per 100 grams ration was sufficient.

Singsen and Mitchell (1945) employed a chick diet containing 0.23 per cent phosphorus, supplemented with either cod liver oil or irradiated animal sterol, and calcium-magnesium phytate to raise the total phosphorus level to 0.49 per cent. Slightly higher bone ash was supported by equivalent unitage of the irradiated product.

THIS RELATIVE DIFFERENCE SUGGESTS THAT THE IRRADIATED PRODUCT MAY HAVE CAUSED EITHER APPRECIABLE UTILIZATION OF THE PHYTATE PHOSPHORUS OR MORE EFFICIENT UTILIZATION OF THE NON-PHYTIN PHOSPHORUS IN THE DIET.

LITTLE HAS BEEN DONE ON THE ABSORPTION AND EXCRETION OF CALCIUM AND PHOSPHORUS BUT SOME INTERESTING EVIDENCE WAS REPORTED BY TYLER (1948). IT WAS REPORTED USING IN VITRO EXPERIMENTS THAT OYSTER SHELL, PURE CALCIUM CARBONATE, BONEMEAL, AND PURE TRICALCIUM PHOSPHATE REMAINED UNDISSOLVED IN A SOLUTION CONTAINING CALCIUM AND PHOSPHORUS AT THE pH OF THE SMALL INTESTINE, AND, AS A SOLID PHASE, REMOVED CALCIUM PARTIALLY AND PHOSPHORUS MORE COMPLETELY FROM SOLUTION. CARBONATES WERE MORE EFFECTIVE THAN PHOSPHATES AND CRUDE COMPOUNDS MORE EFFECTIVE THAN PURE. WHEN THE CONTENTS OF THE EXCISED INTESTINE OF FIVE-WEEK-OLD CHICKS WERE ANALYZED, THE NORMAL RATION SHOWED AN INCREASE OF CALCIUM AND PHOSPHORUS IN THE SOLID PHASE FROM THE UPPERMOST TO THE LOWEST OF THREE EQUAL SECTIONS; THE INCREASE WAS GREATER WITH EXCESS OYSTER SHELL FLOUR OR BONEMEAL. WITH RATIONS HIGH IN MINERALS THE CONCENTRATION OF CALCIUM IN THE SOLID PHASE WAS GREATER THAN NORMAL IN THE UPPER THIRD OF THE SMALL INTESTINE BUT NOT SIGNIFICANTLY DIFFERENT FROM NORMAL LOWER DOWN. WITH OYSTER SHELL, THE CONCENTRATION OF PHOSPHORUS WAS LESS IN THE MIDDLE THIRD OF THE SMALL INTESTINE. THE ABNORMAL COMPOSITION OF THE LIQUID PHASE WHEN OYSTER SHELL WAS GIVEN WAS REFLECTED IN HIGH BLOOD CALCIUM, LOW BLOOD PHOSPHORUS AND BONE ASH.

THE EXCRETION OF INTRAMUSCULARLY ADMINISTERED P^{32} INTO, AND P^{32} CONTENT OF THE STOMACH, DUODENUM, ILEUM, JEJUNUM, CECUM, AND COLON OF FOUR GROUPS OF RATS ON RATIONS CONTAINING MOLYBDENUM AND

COPPER AT LEVELS OF 80 TO 35, 0 TO 35, 80 TO 0, AND ZERO TO ZERO PPM, RESPECTIVELY WERE DETERMINED AT INTERVALS RANGING FROM 0.5 TO 168 HOURS AFTER ADMINISTRATION BY SHIRLEY, OWENS, AND DAVIS (1951). THE RESULTS WERE CALCULATED AS PER CENT DOSE AND PER CENT DOSE PER MILLIGRAM OF TOTAL PHOSPHORUS. THE GROUP FED THE HIGH LEVELS OF BOTH MOLYBDENUM AND COPPER EXCRETED LESS OF THE ISOTOPE INTO THE CONTENTS OF THE VARIOUS ALIMENTARY SEGMENTS THAN THE OTHER THREE GROUPS, WHICH WERE ESSENTIALLY EQUIVALENT WITH RESPECT TO EXCRETION. FECAL AND URINARY EXCRETION VARIED MARKEDLY AMONG THE FOUR GROUPS. THE PER CENT P^{32} IN THE FECES WAS 3.05, 4.54, 5.66, AND 7.74, AND IN THE URINE 12.0, 16.0, 19.3, AND 26.0 FOR THE HIGH COPPER-HIGH MOLYBDENUM, HIGH COPPER, HIGH MOLYBDENUM, AND BASAL RATION FED RATS, RESPECTIVELY, 168 HOURS AFTER ADMINISTRATION.

THE BODY STORES OF CALCIUM AND PHOSPHORUS IN THE BONES ARE LABILE AND CAN ONLY BE FILLED IN THE YOUNG GROWING ORGANISM IF THE CALCIUM AND PHOSPHORUS IN THE DIET BEAR A FAVORABLE RATIO TO ONE ANOTHER AS REPORTED BY OWEN (1952). IN ANIMALS ON DIETS OTHERWISE ADEQUATE BUT DEFICIENT IN CALCIUM, PHOSPHORUS, OR MAGNESIUM, THE SOFT TISSUES COMPETE WITH THE BONES FOR CALCIUM AND PHOSPHORUS OR MAGNESIUM SO THE MINERAL IS LOST FROM THE SKELETON IN ADULTS. IN THE YOUNGER ORGANISM AN UNDERMINERALIZED SKELETON, WHICH MAY BE RICKETY, IS FORMED. VITAMIN D PROTECTS THE ORGANISM BY FAVORING THE BONES AT THE EXPENSE OF SOFT TISSUE. MINERAL IN THE TEETH, HOWEVER, IS IRREVERSIBLY DEPOSITED.

PATRICK AND SCHWEITZER (1952) REPORTED THAT RADIOACTIVE P^{32} FED AS INORGANIC SOUBLE PHOSPHATE IN SMALL AMOUNTS PER CHICK WAS MORE RAPIDLY ABSORBED IN THE PRESENCE THAN IN THE ABSENCE OF VITAMIN D. IN CONJUNCTION WITH A DIET CONTAINING NO INORGANIC PHOSPHORUS, THE

P^{32} WAS ABSORBED AS FAST IN THE PRESENCE OF VITAMIN D_2 AS IN THE PRESENCE OF VITAMIN D_3. THIS FACT SEEMS TO INDICATE THAT THE GENERAL LABILITY OF THE ORGANIC PHOSPHORUS IN THE DIET WAS EQUAL WITH EITHER FORM OF VITAMIN D. SEVERAL WATER-SOLUBLE VITAMINS WERE FOUND TO INFLUENCE THE DEPOSITION OF P^{32} IN BONE. IN VIEW OF THIS FINDING, IT SEEMS LOGICAL TO APPLY SUITABLE RESERVATIONS TO ESTIMATES OF PHOSPHORUS REQUIREMENT IN CASES OF INCOMPLETE DIETS OR OF RELATIVELY POOR GROWTH. ACCORDING TO THESE WORKERS CALCIUM PANTOTHENATE, NIACIN, PARA-AMINO BENZOIC ACID, PYRIDOXINE, VITAMIN B_{12}, AND FOLIC ACID PLAY A PART IN BONE MINERALIZATION.

THE EFFECT OF AUREOMYCIN ON THE MANGANESE REQUIREMENT OF CHICKS FED VARYING LEVELS OF SALT AND PHOSPHORUS WAS REPORTED BY PEPPER, SLINGER AND MOTZOK (1952). THESE EXPERIMENTERS STATED THAT THE REDUCTION IN PERCENTAGE BONE ASH CAUSED BY ADDING AUREOMYCIN TO DIETS CONTAINING 0.41 PER CENT INORGANIC PHOSPHORUS SUGGESTS THAT THIS LEVEL OF PHOSPHORUS MAY NOT BE ADEQUATE FOR MAXIMUM CALCIFICATION IN THE PRESENCE OF AUREOMYCIN.

IT WAS REPORTED BY SIEBURTH AND ASSOCIATES (1952) THAT THE PHOSPHORUS FROM UNIFINE FLOUR (VERY FINELY GROUND WHOLE WHEAT) WAS SHOWN TO BE ALMOST COMPLETELY AVAILABLE FOR GROWTH AND SOMEWHAT LESS AVAILABLE FOR BONE CALCIFICATION IN THE CHICK. ON THE OTHER HAND, FOR OPTIMUM BONE ASH 0.3 PER CENT WHEAT PHOSPHORUS AND 0.4 PER CENT INORGANIC PHOSPHORUS AS SUPPLIED BY DICALCIUM PHOSPHATE WAS REQUIRED. THERE APPEARED TO BE A HIGHER PHOSPHORUS REQUIREMENT FOR OPTIMUM BONE ASH THAN FOR OPTIMUM GROWTH. THE BEST GROWTH REPORTED FROM THIS EXPERIMENT WAS OBTAINED WITH 0.3 PER CENT INORGANIC AND 0.2 PER CENT ORGANIC PHOSPHORUS. IT WAS FURTHER STATED THAT THE ADDITION OF WHOLE

WHEAT FLOUR INCREASED GROWTH AND BONE CALCIFICATION OVER AND ABOVE THAT WHICH COULD BE ACCOUNTED FOR BY PHOSPHORUS ALONE. THE PHOSPHORUS FROM CALCIUM PHYTATE AND ALUMINUM PHOSPHATE WERE RELATIVELY UNAVAILABLE FOR GROWTH ALTHOUGH THAT IN CALCIUM PHYTATE SEEMED TO BE UTILIZED FOR BONE CALCIFICATION AT THE LEVEL USED. THE ADDITION OF ALUMINUM CHLORIDE TO A DIET CONTAINING UNIFINE FLOUR SEEMED TO SOMEWHAT REDUCE THE AVAILABILITY OF PHOSPHORUS FOR GROWTH AND SIGNIFICANTLY REDUCE THE TIBIA ASH.

FISHER AND CO-WORKERS (1953) REPORTED THAT A HIGH AND A LOW ENERGY DIET FOR CHICKS REQUIRED 0.51 PER CENT AND 0.58 PER CENT TOTAL PHOSPHORUS, RESPECTIVELY, FOR MAXIMAL CALCIFICATION AT FOUR WEEKS. THE DIETS CONTAINED A VERY SMALL AMOUNT OF PHYTIN PHOSPHORUS, POSSIBLY 0.08 PER CENT; IF THIS IS SUBTRACTED FROM THE ESTIMATED TOTAL REQUIREMENTS, THE AVAILABLE PHOSPHORUS APPEARS TO BE 0.43 TO 0.50 PER CENT. THESE WORKERS ALSO STATED THAT THERE IS NO INCREASE IN THE ABSOLUTE REQUIREMENT FOR PHOSPHORUS ON A HIGH ENERGY RATION AS COMPARED TO A LOW ENERGY RATION, DESPITE THE APPROXIMATELY 25 PER CENT SMALLER NUTRIENT INTAKE ON THE HIGH ENERGY OVER THE LOW ENERGY RATION; THIS MAY BE EXPLAINED BY THE MUCH GREATER EFFICIENCY OF PHOSPHORUS UTILIZATION.

PHOSPHATIC CLAY (SOFT PHOSPHATE WITH COLLOIDAL CLAY) IS NOT AS GOOD A SOURCE OF PHOSPHORUS AS BONE MEAL OR TRICALCIUM PHOSPHATE, WHEN FED TO CHICKS IN A RATION COMPOSED OF NATURAL INGREDIENTS (GRAU AND ZWEIGART, 1953). ALTHOUGH CLACIFICATION WAS AFFECTED, THE MOST STRIKING EFFECT WAS ON THE GROWTH RATE, WHICH WAS NOT BENEFITED BY THE ADDITION OF PHOSPHATIC CLAY TO THE BASAL DIET. BONE MEAL IMPROVED THE GROWTH RATE. IT WAS FURTHER DEDUCED THAT THE FLUORINE

CONTENT OF PHOSPHATIC CLAY DOES NOT APPEAR TO ACCOUNT FOR ITS EFFECT ON GROWTH. THE DATA OF THESE WORKERS CALCULATED TO INORGANIC PHOSPHORUS LEVELS, INDICATE THAT MAXIMAL TIBIA BONE ASH OF CHICKS WAS OBTAINED AT 28 DAYS OF AGE WITH AN INTAKE OF NOT MORE THAN 0.45 PER CENT PHOSPHORUS.

MILLER AND JOUKOVOSKY (1953) COMPARED PHOSPHORUS SUPPLEMENTS AT TWO DIFFERENT PHOSPHORUS LEVELS USING A TWO TO ONE CALCIUM-PHOSPHORUS RATIO AT BOTH LEVELS. THE PHOSPHORUS FROM DICALCIUM PHOSPHATE, CURACAO ISLAND PHOSPHATE, BONE MEAL, AND DEFLOURINATED PHOSPHATE WERE ALL GOOD SOURCES OF READILY AVAILABLE INORGANIC PHOSPHATE. COLLOIDAL PHOSPHATE WAS A POOR SOURCE OF AVAILABLE PHOSPHORUS FOR GROWING CHICKS AND WOULD SEEM TO HAVE LESS THAN 50 PER CENT OF THE VALUE OF THE OTHER MINERALS TESTED. THESE WORKERS FOUND OF THE VARIOUS PHOSPHATE SUPPLEMENTS FOR CHICKS THAT WERE MADE WITH A BASAL DIET CONTAINING 0.42 PER CENT PHOSPHORUS OF WHICH 0.10 PER CENT WAS PROBABLY READILY AVAILABLE. WITH CHICKS AT FIVE WEEKS OF AGE, PHOSPHATE SUPPLEMENTS WHICH PROVIDED 0.20 PER CENT PHOSPHORUS (0.30 PER CENT TOTAL AVAILABLE PHOSPHORUS) DID NOT PROMOTE MAXIMAL GROWTH OR CALCIFICATION, WHEREAS 0.4 PER CENT ADDED PHOSPHORUS (0.50 PER CENT TOTAL AVAILABLE PHOSPHORUS) APPEARED TO BE SUFFICIENT.

LINBLAD AND ASSOCIATES (1954) REPORTED THAT FEMALE CHICKS SHOWED MAXIMAL GAIN TO SIX WEEKS OF AGE WHEN FED 0.4 PER CENT INORGANIC PHOSPHORUS AS COMPARED TO 0.6 PER CENT IN ASSOCIATION WITH ONE PER CENT CALCIUM IN A PRACTICAL DIET. WITH 1.8 PER CENT CALCIUM THE 0.6 PER CENT LEVEL OF INORGANIC PHOSPHORUS WAS BEST. IN THE CASE OF MALE CHICKS THE 0.6 PER CENT LEVEL OF INORGANIC PHOSPHORUS WAS SUPERIOR

TO 0.4 PER CENT WHEN ASSOCIATED WITH 1.0 OR 1.4 PER CENT CALCIUM. IT WAS CONCLUDED BY THESE WORKERS THAT SOMEWHAT MORE THAN 0.4 PER CENT INORGANIC PHOSPHORUS WOULD BE DESIRABLE IN FEEDING A FLOCK OF MIXED SEXES.

ACCORDING TO CREECH ET AL (1956), BONE ASH DETERMINATIONS, USING BOTH CHICKS AND POULTS, HAVE INDICATED THAT 0.55 PER CENT TOTAL PHOSPHORUS IS SUFFICIENT FOR MAXIMUM BONE CALCIFICATION IN THE CHICK PROVIDED THE SUPPLEMENTAL PHOSPHORUS IS SUPPLIED FROM A SOURCE WHICH CAN BE UTILIZED EFFECTIVELY BY THE CHICK. USING TRICALCIUM PHOSPHATE AS THE SOURCE OF PHOSPHORUS IN A PURIFIED TYPE DIET FOR BOTH CHICKS AND POULTS IT WAS FOUND THAT HIGHER LEVELS WERE REQUIRED TO PRODUCE THE SAME DEGREE OF CALCIFICATION AND GROWTH THAN WHEN DICALCIUM PHOSPHATE WAS USED.

TWENTY-SEVEN PHOSPHATE SUPPLEMENTS, BOTH CHEMICAL AND COMMERCIAL GRADES, WERE TESTED AS TO AVAILABILITY IN A SERIES OF EXPERIMENTS WITH CHICKS OF HEAVY BREEDS TO FIVE WEEKS OF AGE, USING A DIET HAVING A TOTAL PHOSPHORUS CONTENT OF 0.49 PER CENT, (WITH AN INORGANIC PHOSPHORUS CONTENT OF 0.17 PER CENT) BY MOTZOK, ARTHUR AND BRANION, (1956). MOST OF THE COMPARISONS WERE MADE WITH ENOUGH OF EACH OF THE SUPPLEMENTS TO PROVIDE A TOTAL INORGANIC PHOSPHORUS LEVEL OF 0.4 PER CENT, USING TRICALCIUM PHOSPHATE AS A STANDARD. BONE MEAL OR MONOCALCIUM-PHOSPHATE WERE USED AS STANDARDS IN SOME EXPERIMENTS AND LEVELS OF TOTAL INORGANIC PHOSPHORUS, RANGING FROM 0.2 TO 0.8 PER CENT WERE ALSO USED IN SOME EXPERIMENTS. THE CALCIUM:PHOSPHORUS RATIO WAS ADJUSTED TO 2:1 BY THE USE OF CALCIUM CARBONATE. THE ADDITION OF BENTONITE, CHARCOAL, OR CELITE, IN AMOUNTS EQUAL TO THE INERT MATERIAL IN COLLOIDAL PHOSPHATES, TO A DIET SUPPLEMENTED WITH MONOCALCIUM PHOSPHATE, DID NOT DECREASE GROWTH OR PER CENT BONE ASH.

THE ADDITION OF IRON AND ALUMINUM OXIDES, IN AMOUNTS PRESENT IN THE COLLOIDAL PHOSPHATES, WAS ALSO WITHOUT EFFECT. HENCE, THE LOWER AVAILABILITY OF THE PHOSPHORUS IN THE SUPPLEMENTS WAS NOT DUE TO THE "IMPURITIES".

PHEASANT CHICKS WERE FED DIETS CONTAINING 0.66 PER CENT PHOSPHORUS (CHEMICAL ANALYSIS) BY SUNDE AND BIRD (1956), AND HIGH MORTALITY RESULTED. THE PHEASANT CHICKS, AFTER TWO WEEKS ON THIS DIET, SHOWED A DISTINCT BENDING OF THE TARSO-METATARSUS NEAR THE HOCK JOINT AND OF THE PROXIMAL END OF THE TIBIO-TARSUS. BONE ASH VALUES WERE REDUCED FROM A NORMAL OF ABOUT 52 PER CENT TO 39 PER CENT WHEN THE BASAL DIET WAS FED. ADDITIONS OF 0.3 PER CENT TO 0.8 PER CENT PHOSPHORUS FROM DICALCIUM PHOSPHATE RESULTED IN NORMAL GROWTH, NORMAL BONE FORMATION AND CALCIFICATION.

A REVIEW OF ALL EVIDENCE ON READILY AVAILABLE PHOSPHORUS REQUIREMENT FOR THE CHICK TO FOUR WEEKS OF AGE SUGGESTS THAT THIS REQUIREMENT SHOULD BE PLACED AT A MINIMUM OF 0.45 PER CENT, IN THE PRESENCE OF AMPLE AMOUNTS OF VITAMIN D. AFTER THIS AGE THE REQUIREMENT MAY DROP TO 0.37 PER CENT FOR THE PERIOD UP TO TEN WEEKS (ALMQUIST 1954).

AS PHOSPHORUS IS PRESENT NEARLY EVERYWHERE IN THE ANIMAL ORGANISM, SO IT IS PRESENT NEARLY EVERYWHERE IN NATURE. ULTIMATELY, THE SOURCE OF PHOSPHORUS IN THE ANIMAL IS THE SOIL. FOR MAN THE FLOW IS FIRST TO THE PLANT FOODS, THEN EITHER DIRECT TO THE HUMAN ORGANISM OR INDIRECTLY BY WAY OF THE ANIMAL TISSUES CONSUMED BY MAN. IN VIEW OF THE COMPETING DEMANDS FOR PHOSPHORUS TO SERVE ITS MANIFOLD FUNCTIONS IN THE ORGANISM, IT IS IMPORTANT TO NOTE THAT THE FIRST EFFECT OF A DEFICIENT ASSIMILATION OF PHOSPHORUS IS A FAILURE OF CALCIFICATION OF THE BONES AND THAT THIS FAILURE, BY REMOVING THE LARGEST REQUIREMENT FOR THE ELEMENT,

USUALLY LEAVES AN AMOUNT ADEQUATE FOR ALL OTHER PURPOSES, EXCEPT UNDER THE MOST EXTREME EXPERIMENTAL CONDITIONS AS THOSE STATED BY McLEAN AND URIST (1955).

SULFUR

THE BODY CONTAINS APPROXIMATELY 0.15 PER CENT SULFUR AS REPORTED BY MAYNARD AND LOOSLI (1956). THIS ELEMENT OCCURS ALMOST ENTIRELY IN ORGANIC COMPOUNDS, NOTABLY IN PROTEINS IN WHICH IT IS PRESENT AS THE SULFUR-CONTAINING AMINO ACIDS CYSTINE AND METHIONINE. BOTH THE FECES AND URINE ARE PATHS OF SULFUR EXCRETION. IN THE URINE THREE FORMS OCCUR: INORGANIC SULFATES, THE PRINCIPLE FRACTION, WHICH REPRESENTS THE FINAL STAGE OF OXIDATION OF ORGANIC SULFUR; ETHEREAL SULFUR, WHICH IS PRESENT IN COMPLEX DETOXICATION PRODUCTS; AND NEUTRAL SULFUR, WHICH OCCURS AS CYSTINE, TAURINE, THIOSULFATES, AND OTHER COMPOUNDS. SINCE EXCRETED SULFUR ARISES PRIMARILY FROM PROTEIN CATABOLISM, THERE IS A RATHER CONSTANT RATIO BETWEEN IT AND THE NITROGEN IN THE URINE. THERE IS EVIDENCE THAT THE EXCRETION OF NEUTRAL SULFUR IS PROPORTIONAL TO THE BASAL METABOLISM.

MACHLIN AND ASSOCIATES (1954) REPORTED ON CHICKS WHICH WERE FED A CORN-SOYBEAN DIET AND INJECTED INTRAMUSCULARLY WITH 21 MICRO-CURIES OF LABELED L-METHIONINE (0.8 MILLIGRAMS) AT THREE WEEKS OF AGE. SUPPLEMENTARY METHIONINE WAS REMOVED FROM THE DIET TWO DAYS BEFORE INJECTION AND REPLACED ONE DAY AFTERWARDS. BIRDS WERE SACRIFICED AT

FOUR AND EIGHT HOURS AND ONE, THREE, NINE, EIGHTEEN, AND THIRTY SIX DAYS AFTER THE INJECTION. S^{35} ACTIVITY WAS DETERMINED IN THE GIZZARD LINING, SECONDARY WING FEATHERS, LIVER AND LEG MUSCLE. A SECOND EXPERIMENT WAS CONDUCTED TO COMPARE METHIONINE AND SULFATE SULFUR UPTAKE IN FEATHERS, GIZZARD LINING, LIVER AND MUSCLE. CHICKS (FOUR WEEKS OF AGE) WERE INJECTED INTRAMUSCULARLY FOR THREE CONSECUTIVE DAYS WITH LABELED METHIONINE. ANOTHER GROUP WAS INJECTED IN THE SAME WAY WITH CARRIER FREE RADIOACTIVE SODIUM SULFATE. THE RESULTS SHOWED THAT THE MUSCLES RETAINS S^{35} FOR A MUCH LONGER TIME THAN EITHER THE GIZZARD LINING OR LIVER. THE LOSS OF THE LABELED COMPOUNDS IN THE GIZZARD LINING WAS MORE RAPID THAN IN THE LIVER. SINCE THE GIZZARD LINING DOES NOT CONTAIN LIVING CELLS, THE LOSS IS PROBABLY CAUSED BY THE WEARING AWAY OF THE LINING, AND NOT METABOLIC ACTIVITY. IF THE ASSUMPTION IS TRUE, ONE HALF OF THE LINING IN THE YOUNG BIRD IS WORN AWAY EVERY FIVE TO TEN DAYS. THE PRESENCE OF SULFATE IN THE GIZZARD LINING AND THE DEMONSTRATION THAT LABELED SULFATE WAS PREFERENTIALLY TAKEN UP BY THIS TISSUE, MIGHT BE AN INDICATION OF THE PRESENCE OF ESTERIFIED SULFATE SUCH AS CHONDROITIN SULFATE. CYSTEINE MERCAPTIDES ISOLATED FROM HYDROLYZATES OF FEATHERS FROM BIRDS INJECTED WITH LABELED METHIONINE OR SULFATE WERE RADIOACTIVE. THIS DEMONSTRATED THE INCORPORATION OF THE SULFUR OF BOTH METHIONINE AND SULFATE INTO CYSTINE IN THE YOUNG CHICKEN.

IN A LATER REPORT, MACHLIN (1955) REPORTED THAT S^{35} WAS FED AS SODIUM SULFATE TO TWO-WEEK-OLD CHICKS RECEIVING THE BASAL DIET SUPPLEMENTED WITH 0.4 PER CENT METHIONINE FOR A PERIOD OF ONE WEEK. AN AVERAGE OF 23 PER CENT OF THE S^{35} WAS RECOVERED IN THE CARCASS. OF THE AMOUNT RECOVERED, 69 PER CENT WAS PRESENT AS PROTEIN BOUND

SULFATE - S^{35}, FIVE PER CENT AS "FREE" SULFATE - S^{35} AND 19 PER CENT AS TAURINE - S^{35}. THE PROTEIN CONTAINED NEGLIGIBLE AMOUNTS OF RADIOACTIVE CYSTINE OR METHIONINE. IT WAS CONCLUDED THAT THE GROWTH RESPONSE OBTAINED BY THE FEEDING OF SULFATE IS DUE TO A PHYSIOLOGICAL REQUIREMENT PER SE. THIS REQUIREMENT CAN BE SATISFIED BY EITHER THE ADDITION OF SULFATE TO THE DIET OR PRESUMABLY FROM THE SULFATE FURNISHED BY THE OXIDATION OF THE SULFUR-CONTAINING AMINO ACIDS. IF FREE TAURINE HAS SOME PHYSIOLOGICAL FUNCTIONS, THEN SULFATE MIGHT ALSO BE OF VALUE TO THE CHICKEN AS A PRECURSOR OF TAURINE. UTILIZATION OF SULFATE-SULFUR FOR THE SYNTHESIS OF CYSTINE OR METHIONINE IS PROBABLY OF NO NUTRITIONAL SIGNIFICANCE IN THE YOUNG CHICKEN.

GORDON AND CO-WORKERS (1957) PRESENTED EVIDENCE WHICH DEMONSTRATED THAT ALPHA-HYDROXY GAMMA-METHYLMERCAPTO BUTYRIC ACID (THE ALPHA-HYDROXY ANALOGUE OF METHIONINE) IS FULLY EQUIVALENT TO METHIONINE FOR SATISFYING THE REQUIREMENTS OF THE CHICKEN FOR THE SULFUR AMINO ACIDS. EVIDENCE FOR AT LEAST ONE METABOLIC PATHWAY INVOLVED IN THE INTERCONVERSION OF METHIONINE HYDROXY ANALOGUE AND METHIONINE WAS FOUND. THE SYSTEMS STUDIED IN GREATEST DETAIL WERE FRACTIONATED HOMOGENATES OF CHICKEN LIVER. IT WAS FOUND THAT MITACHONDRIAL PREPARATIONS ARE CAPABLE OF OXIDIZING METHIONINE HYDROXY ANALOGUE TO ALPHA-KETO-GAMMA METHYLMERCAPTO BUTYRIC ACID (THE ALPHA KETO ANALOGUE OF METHIONINE) AND THAT WATER SOLUBLE ENZYMES FOUND IN THE SUPERNATANT FRACTIONS AFTER HIGH SPEED CENTRIFUGATION OF LIVER, CONTAIN ENZYMES WHICH RAPIDLY CONVERT METHIONINE KETO ANALOGUE TO METHIONINE HYDROXY ANALOGUE AND METHIONINE. BY MEANS OF DIFFERENTIAL CENTRIFUGATION AND VARIOUS CHEMICAL METHODS, IT WAS POSSIBLE TO PURIFY THE ENZYME SYSTEMS TO AN EXTENT GREAT ENOUGH TO PERMIT DISCOVERY OF THE COFACTORS REQUIRED

IN THIS SYSTEM. THIS WORK SUGGESTS A POSSIBLE MECHANISM FOR THE KNOWN NUTRITIONAL EQUIVALENCE OF THE ALPHA-HYDROXY AND THE ALPHA AMINO COMPOUNDS, AND IN ADDITION PROVIDES FURTHER INFORMATION CONCERNING THE MECHANISM INVOLVED IN THE UTILIZATION OF THE D OR UNNATURAL FORMS OF THESE COMPOUNDS. FEMALE CROSSBRED CHICKS WERE FED A GLUCOSE, CASEIN, GELATIN, FAT, CHOLINE, SULFATE-FREE-MINERAL MIXTURE PURIFIED DIET WHICH WAS FORTIFIED WITH ALL VITAMINS NECESSARY FOR CHICK GROWTH. BOTH WITH AND WITHOUT SUPPLEMENTAL ALPHA-TOCOPHEROL (TEN MILLIGRAMS PER KILOGRAM), THE CHICKS ON THIS DIET DEVELOPED A MUSCULAR DEGENERATION MANIFESTED GROSSLY AS WHITE STRIATIONS OF THE BREAST AND LEG MUSCLES, AND MICROSCOPICALLY AS A HYALINE TYPE DEGENERATION, CLUMPING OF THE FIBERS IN EOSINOPHILIC MASSES AND LOSS OF CROSS STRIATIONS. NO SIGNIFICANT CHANGES WERE NOTED IN THE TISSUES OF THE GIZZARD, HEART, LIVER, OR KIDNEY. ADDITION OF ADEQUATE AMOUNTS OF ALPHA-TOCOPHEROL ACETATE OR 0.5 PER CENT DL-METHIONINE, OR 0.5 PER CENT L-LYSINE, OR 0.25 PER CENT DIPHENYL-P-PHENYLEMEDIAMINE, TO THEIR BASAL DIETS COMPLETELY PREVENTED MUSCULAR DEGENERATION. ADDITION OF INOSITOL, TRYPTOPHAN, BUTYLATED HYDROXY-ANISOLE, OR SODIUM SULFATE HAD LITTLE OR NO EFFECT ON THIS PATHOLOGICAL ALTERATION.

VITAMIN D INCREASES THE INTAKE OF INORGANIC SULFUR AS MEASURED BY DEPOSITION OF S^{35} IN CARTILAGE. CHICKS WHICH RECEIVE A RATION INADEQUATE IN METHIONINE USE A GREATER PERCENTAGE OF SUPPLEMENTARY INORGANIC SULFUR THAN THOSE WHICH RECEIVE ADEQUATE AMOUNTS OF SULFUR-CONTAINING AMINO ACIDS ACCORDING TO PATRICK (1955). VITAMIN B_{12} AND AUREOMYCIN REDUCE THE AMOUNT OF S^{35} WHICH APPEARS IN THE CARTILAGE. THE ROLE PLAYED BY VITAMIN B_{12} AND VITAMIN D WITH INORGANIC SULFUR APPEARS TO BE RELATED TO CHONDROITIN SULFATE.

An experiment which was designed to test the relative growth-stimulating effect of different forms of sulfur in day-old chicks fed a corn-soybean meal type ration was reported by Tsang and Schaible (1957). Materials tested were added at the expense of corn meal. Where calcium sulfate was used, an equivalent amount of calcium was omitted from the ration. The duration of the trial was eight weeks and the birds were grown in battery brooders. The average weights at the end of the trial for chicks fed the basal diet, 0.1 per cent and 0.2 per cent elemental sulfur, 0.55 per cent and 1.1 per cent calcium sulfate (equal to 0.1 and 0.2 per cent elemental sulfur, respectively) and 0.2 per cent methionine were 1012, 1045, 933, 1050, 1033, and 1072 grams, respectively. These data show that the organic form of sulfur (methionine) was the most effective in promoting chick growth on the ration employed. One-tenth per cent elemental sulfur, 0.55 per cent and 1.1 per cent calcium were almost as effective, but 0.2 per cent elemental sulfur depressed growth.

Potassium

In contrast to sodium, the potassium of the body exists primarily as a cellular constituent. Human-blood cells for example, contain over 20 times as much of the element as does plasma. Potassium plays a vital but little understood role in muscle where its content is about six times that of sodium. While blood plasma contains many

TIMES AS MUCH SODIUM AS POTASSIUM, IN MILK THE REVERSE IS TRUE. THE REASON FOR THE VERY DIFFERENT DISTRIBUTION THROUGHOUT THE BODY OF THESE TWO ELEMENTS, WHICH ARE SO CLOSELY RELATED CHEMICALLY, IS A MYSTERY (MAYNARD AND LOOSLI 1956). POTASSIUM DEFICIENCY HAS BEEN EXPERIMENTALLY PRODUCED IN SEVERAL SPECIES. IN ADDITION TO NON-SPECIFIC GROSS SYMPTOMS, THERE IS A LOWERED CONTENT OF THE ELEMENT IN THE HEART AND OTHER ORGANS, HEART LESIONS, TUBULAR DEGENERATION OF THE KIDNEYS AND OTHER PHYSIOLOGICAL CHANGES. AS STUDIED WITH RATS, PIGS, AND CHICKENS THE POTASSIUM REQUIREMENT MARKEDLY EXCEEDS THAT OF SODIUM. REPORTED FIGURES RANGE FROM APPROXIMATELY 0.2 PER CENT TO APPROXIMATELY 0.3 PER CENT OF THE DRY RATION. THE COMMONLY FED RATIONS OF FARM ANIMALS CAN BE COUNTED ON TO MEET THESE REQUIREMENTS. POTASSIUM IS READILY ABSORBED AND THE EXCESS OVER BODY NEEDS IS IMMEDIATELY EXCRETED. THIS EXCRETION NORMALLY TAKES PLACE IN THE URINE TO THE EXTENT OF 90 PER CENT, BUT PROFUSE SWEATING DIVERTS A LARGE PORTION THROUGH THIS CHANNEL.

ACCORDING TO WHITE AND ASSOCIATES (1954) THERE IS NO ADEQUATE EXPLANATION OF HOW CELLS ACQUIRE AND MAINTAIN THEIR POTASSIUM CONCENTRATION. SINCE THIS PROCESS IS OPPOSED BY DIFFUSION, THE STEADY STATE MAINTAINED BY THE CELLS REQUIRES UTILIZATION OF ENERGY OBTAINED FROM OXIDATIONS. ADMINISTERED RADIO-POTASSIUM (K^{42}) IS DIFFERENTIALLY PICKED UP BY THE VARIOUS TISSUES. THE RATE AT WHICH K^{42} ENTERS VARIOUS TISSUES APPEARS TO BE RELATED TO THEIR VASCULARITY RATHER THAN TO THEIR METABOLISM. THE POTASSIUM LOST VIA THE KIDNEYS OR THE GASTROINTESTINAL TRACT IS ACCOMPANIED BY CHLORIDE WHICH MUST COME LARGELY FROM EXTRACELLULAR FLUID. THIS, IN TURN, IS COMPENSATED FOR BY A RISE IN EXTRACELLULAR BICARBONATE CONCENTRATION SO THAT POTASSIUM DEFICIENCY IS

USUALLY ACCOMPANIED BY ALKALOSIS. RESTORATION OF NORMAL EXTRACELLULAR BICARBONATE AND CHLORIDE CONCENTRATIONS IS POSSIBLE ONLY AS A RESULT OF POTASSIUM ADMINISTRATION.

ANALYSES FOR SODIUM AND POTASSIUM WERE CARRIED OUT ON BRAIN, SKIN, MUSCLE, LIVER, AND BLOOD OF CHICKS BY KRAVIS AND KARE (1956). THESE ANALYSES WERE DONE DAILY FOR THE FIRST SEVEN DAYS AFTER HATCHING AND AT LONGER INTERVALS AFTER THIS PERIOD. OBSERVATIONS WERE MADE ON THE RELATIONSHIPS BETWEEN THE CHANGES OF THE CONCENTRATIONS OF SODIUM AND POTASSIUM IN THE VARIOUS TISSUES, AND AT WHAT POINT THE HOMEOSTATIC REGULATORY MECHANISMS OF THE ANIMALS APPEARED TO EXERT A MORE COMPLETE EFFECT. THE RECIPROCAL ADJUSTMENT OF THE ELECTROLYTE LEVELS IN THE MUSCLE WITH AGE WAS STUDIED INTENSIVELY. THE SODIUM LEVEL OF THE PECTORAL MUSCLE WAS OBSERVED TO STEADILY DECREASE TO 15 MILLIEQUIVALENTS 35 DAYS LATER. THERE WAS A RECIPROCAL CHANGE IN THE MUSCLE POTASSIUM FROM 31 MILLIEQUIVALENS ON THE FIRST DAY RISING TO 87 MILLIEQUIVALENTS ON THE THIRTY-FIFTH DAY. THERE APPEARED TO BE LESS VARIATION IN THE VALUES FOR THE INDIVIDUAL SAMPLES AS THE AGE OF THE EXPERIMENTAL ANIMALS ADVANCED.

MINERAL ELEMENT INTERRELATIONS

MOLYBDENUM DEFICIENCY AND TUNGSTATE INHIBITION STUDIES WERE REPORTED BY HIGGINS AND ASSOCIATES (1956B). CRITERIA STUDIED WERE GROWTH RATE, REPRODUCTION, PURINE OXIDATION AND TISSUE LEVELS OF

XANTHINE OXIDASE AND MOLYBDENUM. MOLYBDENUM AND TUNGSTEN WERE ADDED TO DIETS AS SODIUM MOLYBDATE AND SODIUM TUNGSTATE, BUT CONCENTRATIONS WERE RECORDED FOR THE ELEMENT ALONE. THE OCCURRENCE OF THE MORE OBVIOUS DEFICIENCY SYMPTOMS IN THE CHICK MAY BE ATTRIBUTABLE TO A GREATER NEED BY THE CHICK FOR MOLYBDENUM AND XANTHINE DEHYDROGENASE IN THE FORMATION OF LARGE AMOUNTS OF URIC ACID BY THIS SPECIES. CHICKS FED SYNTHETIC DIETS CONTAINING APPROXIMATELY 20 MICROGRAMS MOLYBDENUM PER KILOGRAM DEVELOPED NORMALLY BUT THE ADDITION OF SODIUM TUNGSTATE AT A LEVEL EQUIVALENT TO A TUNGSTEN:MOLYBDENUM RATIO OF 1000 OR 2000:1 (WEIGHT/WEIGHT) PRODUCED AN APPARENT MOLYBDENUM DEFICIENCY. GROWTH RATES WERE DEPRESSED SOMEWHAT AND MORTALITY WAS APPROXIMATELY 25 PER CENT IN FIVE WEEKS. ALL TISSUE XANTHINE DEHYDROGENASE AND MOLYBDENUM CONCENTRATIONS WERE MARKEDLY DEPLETED BY THE TUNGSTATE, AND ABOUT ONE-HALF OF THE URIC ACID NORMALLY EXCRETED BY CHICKS WAS REPLACED BY A MIXTURE OF XANTHINE AND HYPOXANTHINE. ALL OF THE EFFECTS OF THE 1000:1 TUNGSTEN LEVEL WERE COMPLETELY REVERSED BY ADDING AN ADDITIONAL TWO MILLIGRAMS MOLYBDENUM PER KILOGRAM OF DIET. THE MOLYBDENUM REMOVED FROM THE TISSUES BY PROTEIN DEFICIENCY OR TUNGSTATE FEEDING APPEARED IN THE URINE AS A SHARP SPIKE IN MOLYBDENUM EXCRETION; NEITHER DIET INTERFERED WITH THE ABSORPTION OF MOLYBDENUM FROM RAT INTESTINE. NEITHER SODIUM SULFATE NOR SODIUM CHROMATE AFFECTED THE METABOLISM OF MOLYBDENUM. IN ANOTHER REPORT HIGGINS AND ASSOCIATES (1956B) FED CHICKS A SYNTHETIC LOW-MOLYBDENUM RATION CONTAINING 4.5 OR 9.4 MILLIGRAMS PER CENT TUNGSTEN (AS SODIUM TUNGSTATE) DEVELOPED AN APPARENT MOLYBDENUM DEFICIENCY. GROWTH RATES WERE DEPRESSED EIGHT AND 19 PER CENT, RESPECTIVELY IN FIVE WEEKS. LIVER AND OTHER TISSUE MOLYBDENUM CONCENTRATIONS WERE LESS THAN 10 PER CENT OF THE NORMAL VALUES,

WHILE XANTHINE DEHYDROGENASE ACTIVITIES OF THE SMALL INTESTINE, LIVER, KIDNEY, AND PANCREAS WERE REDUCED TO LESS THAN 15 PER CENT OF NORMAL BY BOTH DIETS. ALL OF THESE EFFECTS WERE COMPLETELY REVERSED, AND NORMAL VALUES WERE RESTORED BY ADDING TWO AND SIX MILLIGRAMS PER CENT MOLYBDENUM (AS SODIUM MOLYBDATE), RESPECTIVELY TO THE DIETS CONTAINING TUNGSTATE, EXCEPT THAT WITH THE 9.4 MILLIGRAMS PER CENT DIET, GROWTH WAS STILL DEPRESSED APPROXIMATELY FOUR PER CENT AND MORTALITY WAS NINE PER CENT. WEANLING RATS FED SIMILAR TUNGSTEN-CONTAINING DIETS HAD ESSENTIALLY NO XANTHINE OXIDASE ACTIVITY IN THE SMALL INTESTINE, LIVER, KIDNEY, LUNGS, OR SPLEEN.

IN STUDIES ON THE CONDITIONS REQUIRED TO DEVELOP MOLYBDENUM DEFICIENCY IN CHICKS, EXPERIMENTS WERE CONDUCTED USING CASEIN-CERELOSE DIETS CONTAINING 0.51 TO 0.80 PPM MOLYBDENUM (LEACH AND NORRIS 1957). THE ADDITION OF MOLYBDENUM TO THESE DIETS HAD NO EFFECT ON GROWTH, WHEREAS THE ADDITION OF TUNGSTEN REDUCED GROWTH AND MOLYBDENUM CONTENT OF THE LIVERS. FURTHER STUDIES WERE CONDUCTED USING SOYBEAN PROTEIN-CERELOSE DIETS CONTAINING 1.8 TO 2.1 PPM MOLYBDENUM MOLYBDENUM. TUNGSTEN ADDITION REDUCED GROWTH AND LIVER XANTHINE OXIDASE. MOLYBDENUM WAS EFFECTIVE IN RESTORING XANTHINE OXIDASE ACTIVITY TO NORMAL, BUT ONLY PARTIALLY EFFECTIVE AT THE LEVELS USED IN RESTORING GROWTH. MOLYBDENUM FAILED TO PROMOTE GROWTH IN HATCHERY CHICKS FED SOYBEAN PROTEIN-CERELOSE DIETS. IT ALSO HAD NO BENEFICIAL EFFECT ON THE GROWTH OF CHICKS OBTAINED FROM DAMS FED A DIET CONTAINING REAGENT GRADE MINERALS FOR APPROXIMATELY THREE MONTHS AND GIVEN DEMINERALIZED WATER. THE MOLYBDENUM CONTENT OF THE DIETS OF THE DAMS WAS 1.45 PPM AND 2.8 PPM RESPECTIVELY. STATISTICAL ANALYSIS OF THE DATA INDICATED THAT THE CHICKS RECEIVING MOLYBDENUM GREW AT A SIGNIFICANTLY SLOWER RATE THAN

CHICKS RECEIVING THE ASH OF UNIDENTIFIED-FACTOR SUPPLEMENTS.

KONEN (1958) STATED THAT MOLYBDENUM IS NECESSARY FOR NORMAL GROWTH IN POULTRY. ITS PURPOSE IS TO ALLEVIATE TUNGSTEN TOXICITY. THIS WORKER SUGGEST 0.05 TO 0.1 PPM MOLYBDENUM FOR POULTRY AS THE APPROXIMATE NUTRITIONAL LEVELS. THESE VALUES SHOULD NOT BE CONSIDERED MINIMUM QUANTITATIVE REQUIREMENTS, BUT ARE MORE IN THE NATURE OF OPERATING ALLOWANCES.

DICK AND BULL (1945) WERE FIRST TO EXPERIMENTALLY SHOW THAT THE STORAGE OF COPPER IN THE LIVERS OF SHEEP AND CATTLE COULD BE SIGNIFICANTLY REDUCED BY AN INCREASE IN THE MOLYBDENUM INTAKE. THIS FINDING HAS SINCE BEEN REPEATED BY DICK (1952, 1953A, 1954A). HOWEVER, LEWIS AND ALLCROFT (1953), AND STEWART AND ASSOCIATES (1946) WERE UNABLE TO CONFIRM THE ORIGINAL RESULTS OF DICK AND BULL (1945) AND VARIOUS CONFLICTING AND ANOMALOUS OBSERVATIONS HAVE BEEN MADE WHICH INDICATE THAT THE INHIBITING EFFECT OF MOLYBDENUM ON COPPER STORATE IS A COMPLEX PROCESS INVOLVING DIETARY COMPONENTS OTHER THAN COPPER AND MOLYBDENUM.

NEILANDS AND ASSOCIATES (1948) REPORTED THAT THE TOXICITY OF A RAT DIET CONTAINING 40 MILLIGRAMS PER CENT MOLYBDENUM COULD BE LARGELY OVERCOME BY FEEDING AN ADDITIONAL TWO MILLIGRAMS PER CENT OF COPPER TO A DIET ALREADY CONTAINING 7.73 MILLIGRAMS PER CENT COPPER. OTHER MINERALS SUCH AS IRON, ZINC OR COBALT APPEARED TO BE WITHOUT EFFECT ON MOLYBDENUM TOXICITY BUT WHOLE LIVER SUBSTANCE AFFORDED A MARKED DEGREE OF PROTECTION.

WITH NEWLY WEANED RATS ON A DIET CONTAINING TWO PPM COPPER AND ONE PPM MOLYBDENUM, THE ADDITION OF 80 PPM MOLYBDENUM TO THE DIET SLOWED GROWTH AND GAVE A HIGH MORTALITY RATE. IF 35 PPM

COPPER WERE ADDED AT THE SAME TIME, GROWTH WAS EQUIVALENT TO THAT OF THE CONTROLS AND THE ANIMALS WERE NORMAL IN BEHAVIOR (COMAR ET AL, 1949). USING RADIOACTIVE Mo^{99}, P^{32}, AND Cu^{64}, THE EFFECT OF PHOSPHORUS AND COPPER ON MOLYBDENUM METABOLISM, OF MOLYBDENUM AND COPPER ON PHOSPHORUS METABOLISM, AND OF MOLYBDENUM AND PHOSPHORUS ON COPPER METABOLISM WAS STUDIED IN RATS. THE RESULTS SUGGESTED THAT THE TOXIC ACTION OF MOLYBDENUM MAY BE DUE TO THE FOLLOWING: (1) INTERFERENCE, DUE TO LOW LIVER COPPER, IN ENZYME SYSTEMS NECESSARY FOR SKELETAL METABOLISM, (2) INHIBITION OF THESE ENZYME SYSTEMS BY MOLYBDENUM AND (3) COMPETITION BETWEEN PHOSPHORUS AND MOLYBDENUM FOR DEPOSITION IN BONE. IT IS CONSIDERED UNLIKELY THAT THE TOXIC ACTION OF MOLYBDENUM CAN BE ACCOUNTED FOR SIMPLY BY FORMATION OF A COMPLEX WHICH RENDERS OTHER ELEMENTS UNAVAILABLE.

THE LIMITING EFFECT OF COPPER ON MOLYBDENUM STORAGE WAS SHOWN BY CUNNINGHAM (1950). HE DRENCHED ONE GROUP OF YOUNG CATTLE GRAZING LOW-COPPER, LOW MOLYBDENUM HERBAGE WITH SODIUM MOLYBDATE AT THE RATE OF 150 MILLIGRAMS MOLYBDENUM THREE TIMES PER WEEK. A SECOND GROUP ON THE SAME GRAZING WITH THE SAME LEVEL OF MOLYBDENUM PLUS 100 MILLIGRAMS WAS DRENCHED WITH COPPER THREE TIMES PER WEEK. AFTER SEVERAL MONTHS, THE FIRST GROUP AVERAGED 26 PPM MOLYBDENUM (DRY BASIS) IN THEIR LIVERS COMPARED WITH SEVEN PPM IN THE GROUP RECEIVING BOTH MOLYBDENUM AND COPPER. THE UNTREATED CONTROL GROUPS WERE FOUND TO CONTAIN FIVE PPM MOLYBDENUM IN THEIR LIVERS.

DAVIS (1950) STATED THAT THE HIGH TOLERANCE OF HORSES TO MOLYBDENUM IS ILLUSTRATED BY THEIR FAILURE TO SHOW ANY TOXICITY ON TART PASTURES AND THAT OF PIGS BY A GROUP WHICH WERE FED LEVELS OF MOLYBDENUM AS HIGH AS 1,000 PPM FOR A PERIOD OF NINETY DAYS WITHOUT

ANY ILL EFFECTS. IN CONTRAST TO THE RESULTS WITH PIGS, ARRINGTON AND DAVIS (1953) REPORTED THAT THE INTAKE OF 1,000 PPM OF MOLYBDENUM AS SODIUM MOLYBDATE INDUCED GROSS TOXIC SYMPTOMS IN WEANLING RABBITS WHEN THE DIET CONTAINED 2.7 PPM MOLYBDENUM AND 16.4 PPM COPPER, WHEREAS LEVELS OF 140 PPM AND 500 PPM MOLYBDENUM HAD NO DISCERNIBLE EFFECT. THE EFFECTS OF VARIOUS LEVELS OF MOLYBDENUM ON WEANLING RATS APPEAR TO BE VERY VARIABLE DEPENDING ON THE BASAL DIET FED AND PARTICULARLY UPON ITS COPPER CONTENT. IT WAS FOUND THAT THE ADDITION OF AS LITTLE AS 80 PPM MOLYBDENUM TO A DIET CONTAINING TWO PPM COPPER INHIBITED GROWTH AND INDUCED A HIGH MORTALITY IN WEANLING RATS WHICH COULD BE COMPLETELY OVERCOME BY RAISING THEIR COPPER INTAKE BY ADDITIONS OF COPPER SULFATE TO THE EQUIVALENT OF 35 PPM.

THE EFFECT OF MOLYBDENUM ON COPPER STORAGE IN STALLED SHEEP WAS REPORTED BY LEWIS AND ALLCROFT (1953). THE RESULTS INDICATED THAT LIVER COPPER STORAGE IN THE EWE AND FETUS IS CONTROLLED BY THE NATURE OF THE DIET RATHER THAN BY THE TOTAL COPPER AND/OR MOLYBDENUM INTAKE. IT WAS FURTHER FOUND THAT KALE FED TO EWES DURING PREGNANCY HAS AN EXIBITING EFFECT ON FETAL LIVER COPPER STORAGE AND THAT THE OCCURRENCE OF ATAXIA IN LAMBS IS NOT SOLELY DEPENDENT ON DEPLETION OF COPPER IN EWES AND LAMBS.

USING LYSINE-DEFICIENT AND LYSINE-SUPPLEMENTED DIETS, VOHRA AND KRATZER (1957) REPORTED THAT THE ADDITION OF 2400 PPM COPPER TO THESE DIETS KILLED ALL TURKEY POULTS THAT CONSUMED THESE DIETS. IT WAS FURTHER INDICATED THAT CONTRARY TO THE FINDING IN RATS, A DEFICIENCY OF COPPER DOES NOT CAUSE DEPIGMENTATION IN TURKEY POULTS. THIS WAS INDIRECTLY CONFIRMED BY THE FACT THAT THE ADDITION OF MOLYBDENUM TO LYSINE-SUPPLEMENTED DIETS CAUSED NO DEPIGMENTATION.

THE FEEDING OF 1000 PPM MOLYBDENUM IN THE DIET TO THE POULTS WOULD HAVE INTERFERED WITH THE UTILIZATION OF COPPER PRESENT IN THE DIET AND WOULD HAVE CAUSED COPPER DEFICIENCY SYNDROMES. THE ONLY NOTICEABLE EFFECT OF THE EXCESS MOLYBDENUM WAS A SLIGHT REDUCTION IN GROWTH.

DATA OF DICK (1953A, 1953B, 1954A) HAS SHOWN THAT THE LEVEL OF INORGANIC SULFATE IN THE DIET IS OF MAJOR IMPORTANCE ESPECIALLY IN SHEEP. ONLY IN THE PRESENCE OF ADEQUATE INTAKES OF INORGANIC SULFATE IS THE LIMITING EFFECT OF MOLYBDENUM ON LIVER-COPPER STORAGE EXERTED. IT WAS FURTHER STATED BY THIS AUTHOR THAT, FOR A GIVEN INTAKE OF MOLYBDENUM, THE LIMITATION OF COPPER STORAGE WAS PROPORTIONAL TO THE SULFATE CONTENT OF THE DIET. HE FURTHER CONCLUDED THAT MUCH FURTHER WORK IS NECESSARY BEFORE THE EXACT QUANTITATIVE RELATIONSHIP AMONG COPPER, MOLYBDENUM AND INORGANIC SULFATE INTAKES CAN BE EVALUATED, ALTHOUGH IT APPEARS PROBABLE THAT MANY OF THE APPARENT CONFLICTING REPORTS ON THE EFFECT OF MOLYBDENUM ON COPPER STORAGE MAY BE EXPLAINED ON THE BASIS OF DIFFERENT LEVELS OF INORGANIC SULFATE IN THE DIETS EMPLOYED.

SOME EFFECTS OF EXCESS MOLYBDENUM ON THE NUTRITION OF THE RAT WERE STUDIED BY GRAY AND DANIEL (1954). THE FOUND THAT THE ADDITION OF METHIONINE TO THE DIET MARKEDLY PREVENTED THE HARMFUL EFFECTS OF EXCESS MOLYBDENUM. THE RELATIONSHIP BETWEEN COPPER AND MOLYBDENUM WAS DEMONSTRATED, AS SUPPLEMENTARY COPPER REDUCED BY APPROXIMATELY ONE-THIRD OF THE GROWTH DEPRESSING EFFECT OF MOLYBDENUM FED AT HIGH LEVELS. SUPPLEMENTATION OF THE DIET WITH BOTH METHIONINE AND COPPER WAS NOR MORE EFFICACIOUS IN COUNTERACTING THE TOXICITY THAN THE USE OF METHIONINE ALONE.

MILLER AND PRICE (1956) FOUND THAT THE ADDITION OF 75 OR 100 PPM MOLYBDENUM TO A DIET WHICH CONTAINED NO INORGANIC SULFATE CAUSED

A GROWTH DEPRESSION IN THE RAT. THIS TOXIC ACTION OF MOLYBDENUM COULD BE ALLEVIATED BY INCLUSION IN THE DIET OF 2,200 PPM SULFATE (EITHER SODIUM OR POTASSIUM SULFATE). SIMILAR FINDINGS WERE REPORTED BY VAN REEN AND WILLIAMS (1956).

DAVIES AND ASSOCIATES (1957) REPORTED THAT THE INCLUSION OF 6,000 PPM MOLYBDENUM IN A PURIFIED DIET CAUSED AN 80 PER CENT DEPRESSION IN THE GROWTH OF FOUR-WEEK-OLD CHICKS, WHEN COMPARED TO CHICKS IN THE CONTROL GROUP, BUT DID NOT INCREASE MORTALITY. AN ANEMIC CONDITION WAS FOUND TO BE ASSOCIATED WITH THE FEEDING OF MOLYBDENUM IN EXCESS OF 2,000 PPM. ADDITION OF SODIUM SULFATE TO THE DIET CONTAINING SUPPLEMENTAL MOLYBDENUM AT A LEVEL OF TWO PARTS OF SULFATE TO ONE PART MOLYBDENUM PARTIALLY OVERCAME THE GROWTH DEPRESSING EFFECT CAUSED BY THE FEEDING OF THE SAME LEVEL OF MOLYBDENUM IN THE ABSENCE OF THE SODIUM SULFATE. HOWEVER, THE ALLEVIATING EFFECT OF SULFATE ON THE ANEMIA, FOUND TO ACCOMPANY HIGH MOLYBDENUM INTAKE LEVELS, WAS LESS PRONOUNCED.

GRAY AND ELLIS (1950) REPORTED THAT HIGH LEVELS OF MOLYBDENUM RETARDED GROWTH IN RATS AND THAT HIGH CONCENTRATIONS OF ZINC PRODUCED AN ANEMIA. THE ABILITY OF COPPER TO CORRECT THIS EFFECT OF MOLYBDENUM WAS NOT CLEARLY SHOWN, BUT THE COPPER DID CORRECT THE ANEMIA FROM HIGH LEVELS OF ZINC.

DANIEL AND GRAY (1953) REPORTED THAT LARGE AMOUNTS OF MOLYBDENUM, 40 TO 80 MILLIGRAMS PER TEN MILLILITERS MEDIUM, INHIBITED THE GROWTH OF LACTOBACILLUS LEICHMANNII, BUT THE INHIBITION WAS RAPIDLY OVERCOME BY RELATIVELY SMALL DOSES OF VITAMIN B_{12}. COBALT ALONE DID NOT HAVE THIS EFFECT. ADDITION OF CYSTEINE, GLUTATHIONE, OR BAL (2,3-DIMERCAPTOPROPANOL) TO THE MEDIUM DECREASED THE INHIBITION CAUSED BY

MOLYBDENUM. IT WAS SUGGESTED THAT THIS INHIBITION WAS DUE TO FORMATION OF MOLYBDATE MERCAPTIDES.

MOLYBDENUM ADDED TO CATTLE RATIONS AT LEVELS OF 200 AND 400 PPM MARKEDLY CHANGED THE DISTRIBUTION OF VITAMIN B_{12} IN LIVER AND HEART TISSUES ACCORDING TO DAVIS ET AL (1956). ORALLY ADMINISTERED Co^{60} WAS INCORPORATED INTO VITAMIN B_{12} IN THE RUMEN AT GREATLY DIFFERING RATES DEPENDING ON THE MOLYBDENUM LEVEL OF THE RATION. AT HIGH LEVELS OF MOLYBDENUM VERY LITTLE VITAMIN B_{12} WAS FORMED. BENEFICIAL RESPONSE, IN FIELD TRIALS, TO ADDED COBALT WHEN THE FEED CONTAINED HIGH LEVELS OF MOLYBDENUM APPEAR TO BE ASSOCIATED WITH INCREASED VITAMIN B_{12} SYNTHESIS FOLLOWING INCREASED COBALT CONSUMPTION.

THE OMISSION OF RIBOFLAVIN AND/OR MOLYBDENUM FROM A 24 PER CENT CASEIN DIET FED TO WEANLING RATS HAD NO EFFECT ON LIVER XANTHINE OXIDASE WITHIN TWO WEEKS; A MOLYBDENUM DEFICIENCY IN THE PRESENCE OR ABSENCE OF RIBOFLAVIN REMOVED XANTHINE OXIDASE FROM THE INTESTINE ALMOST COMPLETELY, WHILE A RIBOFLAVIN DEFICIENCY IN THE PRESENCE OF MOLYBDENUM GAVE LEVELS OF INTESTINAL ENZYME WHICH WERE ABOUT ONE-HALF THE SATURATION LEVELS ACHIEVED BY A DIET CONTAINING BOTH MOLYBDENUM AND RIBOFLAVIN (RICHERT AND WESTERFELD, 1953A).

SUMMARY

SUFFICIENT EVIDENCE HAS BEEN PRESENTED TO INDICATE THAT MOLYBDENUM MAY BE AN ESSENTIAL TRACE ELEMENT IN PLANTS AND ANIMALS. IN ANIMALS MOLYBDENUM APPEARS TO BE ABSORBED, ESPECIALLY IN THE HEXAVALENT WATER SOLUBLE FORM, FROM THE INTESTINE AND THE MAIN PATHWAY OF EXCRETION IS THROUGH THE URINE. IT HAS BEEN FOUND THAT SUPPLEMENTAL LEVELS OF LESS THAN ONE PPM HAVE INDUCED GROWTH STIMULATION IN CHICKS AND TURKEY POULTS WHEN FED IN DIETS. MOLYBDENUM HAS BEEN IDENTIFIED AS A COMPONENT PART OF THE ENZYMES NITRATE REDUCTASE, XANTHINE OXIDASE AND ALDEHYDE OXIDASE. SUPPLEMENT OF MOLYBDENUM IN DIETS OF CHICKS AND RATS HAS REPORTEDLY INCREASED THE XANTHINE OXIDASE (DEHYDROGENASE) LEVELS IN LIVERS OF CHICKS AND RATS. ALDEHYDE OXIDASE APPEARS TO BE INDEPENDENT OF DIETARY MOLYBDENUM. THE TOXIC LEVELS OF THIS ELEMENT APPEAR TO VARY IN ANIMALS WITH THE GENUS AND SPECIES.

COPPER HAS BEEN CONSIDERED AN ESSENTIAL DIETARY COMPONENT FOR OVER THREE DECADES. SEEMINGLY THERE IS LITTLE KNOWN ABOUT THE MECHANISM OF ABSORPTION OF THIS ELEMENT IN THE BODY. VARIOUS ENZYMES SUCH AS TYROSINASE, LACCASE, ASCORBIC ACID OXIDASE, AND BUTYRL Co A DEHYDROGENASE HAVE BEEN FOUND TO CONTAIN COPPER. THERE IS DISAGREEMENT REGARDING THE ANIMAL'S ABILITY TO STORE COPPER, ALTHOUGH EVERY ORGAN REPORTEDLY CONTAINS COPPER IN VARYING AMOUNTS. ANEMIA HAS BEEN ATTRIBUTED TO A COPPER DEFICIENCY. SOME RECORD THE TOXIC LEVEL OF THIS ELEMENT IN THE CASE OF GROWING CHICKS AS IN EXCESS OF 1200 PPM WHEN ADDED TO THE RATION.

LITTLE HAS BEEN DONE ON ANIMAL ABSORPTION OF PHOSPHORUS AND POTASSIUM. IT HAS BEEN REPORTED THAT THE BODY'S STORES OF CALCIUM AND PHOSPHORUS ARE LABILE AND CAN BE FILLED ONLY IN YOUNG ORGANISMS AND THEN ONLY WHEN IN A PROPER RATIO WITH CALCIUM. IN CASES OF PHOSPHORUS DEFICIENCY THERE IS REPORTEDLY COMPETITION BETWEEN THE SOFT TISSUES AND BONE. RESULTS HAVE BEEN PRESENTED WHICH SUGGEST THAT PHOSPHORUS IS MORE READILY ABSORBED IN THE PRESENCE OF VITAMIN D. VARIOUS WATER SOLUBLE VITAMINS HAVE BEEN FOUND TO INFLUENCE THE DEPOSITION OF RADIOACTIVE PHOSPHORUS IN BONE. THE PHOSPHORUS REQUIREMENT FOR CHICKS HAS BEEN SUGGESTED AS 0.45 PER CENT OF THE RATION FOR YOUNG CHICKS DECREASING TO 0.35 PER CENT FOR GROWING PULLETS.

THE HUMAN BODY IS SAID TO CONTAIN 0.15 PER CENT SULFUR IN ORGANIC FORM I.E. METHIONINE AND CYSTINE. THE PATHWAY OF EXCRETION IS THROUGH THE URINE IN WHICH THIS ELEMENT IS EXCRETED IN VARIOUS FORMS. THERE HAS BEEN EVIDENCE PRESENTED THAT INORGANIC SULFATE STIMULATES GROWTH IN CHICKS AND SUGGESTS THERE MIGHT BE A SULFATE REQUIREMENT PER SE.

THE POTASSIUM IN THE BODY EXISTS AS A CELLULAR CONSTITUENT. REPORTEDLY THERE IS NO LOGICAL EXPLANATION AS TO HOW CELLS ACQUIRE AND MAINTAIN THE CONCENTRATION OF THIS ELEMENT. POTASSIUM PLAYS A VITAL ROLE IN MUSCLES. EXCESS POTASSIUM IS SAID TO BE EXCRETED BOTH THROUGH THE URINE AND THROUGH THE SWEAT GLANDS. AN ANIMAL REQUIREMENT FOR POTASSIUM IS CONSIDERED TO BE FILLED FROM COMMON DIETS WITH NO SUPPLEMENTATION REQUIRED.

VARIOUS RELATIONSHIPS BOTH SYNERGISTIC (COPPER AND SULFUR) AND ANTAGONISTIC (MOLYBDENUM AND TUNGSTEN; MOLYBDENUM AND COPPER) HAVE BEEN REVIEWED.

EXPERIMENTAL

A. Studies With Deutectomized Chicks

In an attempt to secure molybdenum-free chicks, various methods have been used. If there were a carry-over of this element from the dam to the offspring, it was deduced that this would be found primarily in the unabsrobed yolk material. Deutectomy or surgical removal of the unabsrobed yolk should reduce the amount of molybdenum in the chick and thus result in chicks low in molybdenum stores and consequently a more critical assay animal. This operation was done in the manner reported by Harvey and associates (1955) and care and feeding done as prescribed by Denton et al (1950), Spector (1948), and Spector et al (1950). Williams and DeBusk (1955) reported that is was evident from their experimentas that newly hatched baby chicks do not have substantial stores of many nutrients and immediately depend on the food they consume to suppley every item. Using very accurate weighing of both feed and water consumed and the carcass gain these workers reported good results, especially for a single vitamin and/or amino acid deficiency, by evaluating rations in a brief period of time, viz some as short as a 12 hour duration and others at one or two days. With these facts in mind the operation was performed on 36 newly-hatched chicks. Another group of chicks of equal size, unoperated, was used as controls. Individual feed and water consumption was recorded for 24, 48, and 72 hours. A slurry was made using one gram of the practical corn-soybean meal type broiler ration (shown in Table 1)

AND TWO MILLILITERS OF WATER. EACH MILLILITER OF SLURRY CONTAINED 0.38 GRAM OF FEED AND 0.76 MILLILITER OF WATER. THE CHICKS WERE FORCED-FED USING A HYPODERMIC SYRINGE WITH A LATEX RUBBER TUBE.

TABLE I

PRACTICAL TYPE CHICK DIET USED

INGREDIENT	PER CENT
YELLOW CORN MEAL	58.50
SOYBEAN MEAL (50% PROTEIN)	27.40
MENHADEN FISH MEAL	3.00
ALFALFA LEAF MEAL	3.00
DICALCIUM PHOSPHATE	2.00
OYSTER SHELL FLOUR	1.00
SODIUM CHLORIDE	0.50
ANIMAL FAT	4.00
BACIFERM 10	0.05
DAWES FORBEE	0.15
VITAMIN B_{12} SUPPLEMENT (12 MG/LB)	0.05
VITAMIN A (10,000 U.S.P./LB)	0.053
VITAMIN D (20,000 I.U./LB)	0.012
VITAMIN E (20,000 I.U./LB)	0.025
CHOLINE CHLORIDE	0.160
METHIONINE	0.050
NICARBAZINE	0.050
TOTAL	100.000

Feeding rates for various groups were ad libitum, and in the case of the forced-fed chicks 1.52, 3.04, and 4.56 grams per day. The daily water intake was ad libitum, and in the case of the forced-fed chicks 3.04, 6.08, and 9.12 milliliters, respectively. The forced-fed chicks were dosed every four hours for 16 hours per day. The lights were turned off for the remaining eight hours. The results of this study are presented in Table II.

TABLE II

Results Obtained From Short Term Feeding Trial

Normal Chicks (Gain in Grams)

Daily Feed Intake	24 Hour Gain Lot			48 Hour Gain Lot			72 Hour Gain Lot		
	1	2	3	1	2	3	1	2	3
Ad Lib	3	4	4	8	12	8	21	13	18
1.52 gm	0	0	0	0	-2	2	-2	0	0
3.04 gm	3	3	2	2	2	3	5	5	3
4.56 gm	4	5	5	6	5	6	8	9	6

Deutectomized Chicks

Daily Feed Intake	24 Hour Gain Lot			48 Hour Gain Lot			72 Hour Gain Lot		
	1	2	3	1	2	3	1	2	3
Ad Lib.	-1	-2	-2	5	0	-2	9	0	-5
1.52 gm	1	1	0	-1	0	0	2	0	-1
3.04 gm	4	2	2	5	2	3	2	6	4
4.56 gm	5	1	5	4	1	4	4	10	7

None of the statistical procedures of analysis in any of the studies necessarily treat the data as being orthogonal. Statistical analyses of variance as described by Snedecor (1956) were made of this study and revealed highly significant differences occurring between treatments within (a) time (i.e. 24, 48 and 72 hour feeding periods), (b) condition (normal versus deutectomized) and (c) Feeding levels used. This analysis of variance is shown in Table III

TABLE III

Analysis of Variance on Gain Obtained in Short-Term Feeding Experiment

Source	Degrees of Freedom	Mean Square	F Value
Total	23		
Within Time (24, 48 and 72 hour Feeding periods)	2	63.40	12.43**
Normal vs Deutectomized chicks	1	120.10	23.55**
Between Feeding Levels	3	117.30	23.00**
Error	17	5.11	

** Significant at 0.01 level of probability

Further analyses of variance on the groups of normal chicks revealed the following: At 24 hours the ad libitum method of feeding was found to be highly significantly superior to the 1.52 gram daily intake but not statistically different from the 3.04 or 4.56 gram daily intake; the 4.56 gram daily intake proved to be highly significantly superior to the 1.52 gram daily intake and statistically

SIGNIFICANTLY SUPERIOR TO THE 3.04 GRAM DAILY INTAKE.

AT 48 HOURS AND 72 HOURS, THE AD LIBITUM METHOD OF FEEDING WAS FOUND TO BE HIGHLY SIGNIFICANTLY SUPERIOR TO ALL THREE LEVELS OF FORCED-FEEDING. IN EACH CASE, THE 4.56 GRAM DAILY INTAKE LEVEL WAS HIGHLY SIGNIFICANTLY SUPERIOR TO THE 3.04 GRAM LEVEL, WHICH IN TURN WAS HIGHLY SIGNIFICANTLY SUPERIOR TO THE 1.52 GRAM DAILY INTAKE LEVEL OF RATION.

IN THE CASE OF THE DEUTECTOMIZED CHICKS IT WAS FOUND THAT AFTER 24 HOURS THE 4.56 GRAM DAILY INTAKE LEVEL WAS SUPERIOR, BUT NOT SIGNIFICANTLY, TO THE 3.04 GRAM LEVEL; HOWEVER, THIS HIGHEST LEVEL OF FORCED-FEEDING WAS HIGHLY SIGNIFICANTLY SUPERIOR TO BOTH THE 1.52 GRAM DAILY INTAKE LEVEL AND THE AD LIBITUM FEEDING. IT IS BELIEVED THE REASON THAT AD LIBITUM FEEDING DID NOT HAVE THE SAME EFFECT AS WITH THE NORMAL CHICKS IS THAT THE OPERATION WAS A RATHER SEVERE SHOCK TO THE BIRDS AND THAT A LONGER PERIOD WAS REQUIRED TO OVERCOME THIS SHOCK.

AFTER PERIODS OF 48 AND 72 HOURS, THERE WAS FOUND TO BE NO STATISTICALLY SIGNIFICANT DIFFERENCES IN ANY OF THE FEEDING METHODS. THE DATA PRESENTED IN TABLE II REVEALS CONSIDERABLE DIFFERENCES IN RATES OF GAIN BUT DUE TO THE LARGE AMOUNT OF VARIATION WITHIN EACH FEED LEVEL, THE APPARENT DIFFERENCES ARE NOT STATISTICALLY SIGNIFICANT.

IT WAS APPARENT THAT AD LIBITUM FEEDING WOULD NOT ADEQUATELY SUPPORT GAIN IN AN EXPERIMENT OF SUCH SHORT DURATION. FORCED-FED CHICKS, ESPECIALLY THOSE FED AT HIGHER LEVELS WERE FOUND TO HAVE "PACKED CROPS", AND THE WEIGHTS PROBABLY DID NOT ADEQUATELY REFLECT CARCASS GAIN. ON POST-MORTEM EXAMINATIONS IT WAS ALSO OBSERVED THAT, IN MANY CASES, SEVERE GULLET LESIONS WERE FOUND WHICH WERE DUE TO

THE FORCING THE SMALL RUBBER TUBE INTO THE CROP OF THESE YOUNG CHICKS. THIS APPROACH TO THE PROBLEM OF OBTAINING MOLYBDENUM DEFICIENT CHICKS AND USING THIS SHORT TERM METHOD FOR ATTEMPTING TO DETERMINE GAIN DIFFERENCES WAS ABANDONED AT THIS POINT.

Summary

SHORT TERM EXPERIMENTAL STUDIES WERE MADE WITH DEUTECTOMIZED AND CONTROL CHICKS, IN WHICH AD LIBITUM AND A FORCED-FEEDING TECHNIQUE WAS USED. IT WAS FOUND THAT THE DEUTECTOMIZED CHICKS GREW AT A SIGNIFICANTLY SLOWER RATE THAN DID THE CONTROL CHICKS REGARDLESS OF THE METHOD OF FEED INTAKE. ALTHOUGH SIGNIFICANT DIFFERENCES WERE FOUND BETWEEN THE FEEDING LEVELS IN BOTH THE DEUTECTOMIZED AND CONTROL CHICKS, IT WAS FELT THAT THIS TYPE OF PLAN WOULD NOT BE A FEASIBLE METHOD FOR MOLYBDENUM STUDIES.

B. Tungstate Supplementation of Breeder Hens

In an effort to obtain molybdenum dificient chicks, it was decided to add a reported molybdenum antagonist such as sodium tungstate (Higgins et al 1956a,b, Leach and Norris 1957, and Konen 1958) to the diet of breeder hens. It was felt that this method would yield chicks sufficiently depleted so that they could be used satisfactorily for molybdenum assay.

The purpose of this study was to determine the effect of supplemental tungsten (as sodium tungstate) in the diets of breeder hens.

Sixty White Plymouth Rock hens were obtained and divided into two groups. One group, consisting of 25 hens was fed a corn-soybean meal type breeder ration (Table IV) and the other group of 35 hens was fed this same ration which was supplemented with 250 parts per million (hereafter referred to as ppm) tungsten, as sodium tungstate. Four males were placed with the first group and five with the latter group, with ten males in reserve which were fed a molybdenum supplemented ration. The males in the tungstate fed pens were changed every three days. This was done in order to insure maximum fertility of eggs from hens fed the tungsten supplemented diet. Eggs were collected by trap nest for the duration of the trial. In order to determine the effect of the sodium tungstate, it was decided that periodically a hen would be sacrificed and xanthine dehydrogenase concentration would be determined on the liver, kidney and the intestine (a segment directly posterior to the duodenal loop was used). The Warburg Manometric technique described by Umbriet et al (1957), Remy et al (1951), Remy and Westerfeld (1951), Richert and Westerfeld (1953a,b), and De Renzo et al (1953c), using improved manometric fluid as described by

TABLE IV

PRACTICAL TYPE HEN BREEDER RATION

INGREDIENT	PER CENT
YELLOW CORN MEAL	45.50
SOYBEAN MEAL (50% PROTEIN)	22.00
GROUND OATS	20.00
DICALCIUM PHOSPHATE	4.00
OYSTER SHELL FLOUR	2.00
SODIUM CHLORIDE	1.00
MENHADEN FISH MEAL	2.50
ALFALFA LEAF MEAL	2.50
BACIFER 10	0.05
DAWES FORBEE	0.15
VITAMIN B_{12} SUPPLEMENT (12 MG/LB)	0.05
MANGANESE SULFATE	0.024
VITAMIN E (20,000 I.U./LB)	0.012
VITAMIN D (20,000 I.U./LB)	0.025
VITAMIN A (10,000 U.S.P./LB)	0.083
CHOLINE CHLORIDE	0.160
TOTAL	100.00

Krebs (1951) was used in the determinations. Michaelis' Universal buffer, pH 7.4 was used as the medium with 0.05 milliliters of 0.05 molar xanthine, dissolved in 0.05 molar sodium hydroxide, as the substrate. The tissue dilution were one part tissue to ten parts medium for liver and kidney and one part tissue to five parts medium for the intestine. An incubation period of 40 minutes was allowed at which time the substrate was tipped in, and readings taken. The final readings were made 20 minutes after the initial reading and the endogenous oxygen consumption was routinely determined on a flask which did not contain the substrate.

Xanthine dehydrogenase levels were expressed in cubic millimeters of oxygen consumed per 20 minutes per 100 milligrams of fresh tissue. Tissue dry weights were not used since Reny and Westerfeld (1951) reported that calculation of xanthine dehydrogenase activities on a dry weight basis did not alter the comparative results. The results of these determinations are presented in Table V.

After 10 days on this tungstate supplemented ration it did not appear that any significant changes had resulted in the xanthine dehydrogenase levels. It was then decided to double the supplemental level of tungsten, that is, the addition of 500 ppm tungsten. The manometric method of determining the enzyme activity was continued. The results are shown in Table VI.

Since the xanthine dehydrogenase values were extremely low after a period of 30 days, it was felt that this should be a sufficient length of time to "deplete" these hens of molybdenum. If this were true the progeny hatched from the hens would be expected to be molybdenum deficient.

TABLE V

XANTHINE DEHYDROGENASE LEVELS OF VARIOUS TISSUES FROM HENS CONSUMING FEED CONTAINING 250 PPM SUPPLEMENTAL TUNGSTEN

DAYS FED RATION	TISSUE	DILUTION OF TISSUE	XANTHINE DEHYDROGENASE ACTIVITY* PER FLASK	PER 100 MG TISSUE	PER MG N IN SAMPLE
0	LIVER	1:10	45.68	26.87	-
	KIDNEY	1:10	41.32	24.30	-
	INTESTINE	1:5	27.99	8.23	-
0	LIVER	1:10	87.04	51.20	20.98
	KIDNEY	1:10	74.92	44.07	20.78
	INTESTINE	1:5	30.51	15.25	8.59
1	LIVER	1:10	67.73	39.84	-
	KIDNEY	1:10	40.37	23.73	-
	INTESTINE	1:5	26.84	7.89	-
3	LIVER	1:10	70.27	41.29	12.20
	KIDNEY	1:10	57.81	34.01	9.16
	IMTESTINE	1:5	18.35	9.17	1.56
5	LIVER	1:10	79.46	46.74	13.36
	KIDNEY	1:10	53.92	31.72	8.10
	INTESTINE	1:5	18.08	9.04	1.34
7	LIVER	1:10	84.09	49.47	15.04
	KIDNEY	1:10	41.00	24.12	7.27
	INTESTINE	1:5	18.08	9.04	1.34
10	LIVER	1:10	82.70	48.64	12.41
	KIDNEY	1:10	69.99	41.17	9.87
	INTESTINE	1:5	10.50	5.25	1.03

* XANTHINE DEHYDROGENASE ACTIVITIES ARE RECORDED IN C. MM. OF O_2 CONSUMED PER 20 MINUTES.

ALL VALUES REPRESENT DUPLICATE DETERMINATIONS FOR EACH TISSUE.

TABLE VI

XANTHINE DEHYDROGENASE LEVELS ON VARIOUS TISSUES FROM HENS CONSUMING FEED CONTAINING 500 PPM SUPPLEMENTAL TUNGSTEN

DAYS FED RATION[1]	TISSUE	DILUTION OF TISSUE	XANTHINE DEHYDROGENASE ACTIVITY* PER FLASK	PER 100 MG TISSUE	PER MG N IN SAMPLE
3	LIVER	1:10	96.06	56.60	18.09
	KIDNEY	1:10	43.65	25.68	8.99
	INTESTINE	1:5	29.25	14.62	1.78
6	LIVER	1:10	69.69	40.99	11.71
	KIDNEY	1:10	58.77	34.42	10.74
	INTESTINE	1:5	6.21	3.11	0.91
8	LIVER	1:10	81.52	47.95	14.15
	KIDNEY	1:10	48.74	28.67	9.37
	INTESTINE	1:5	8.50	4.25	1.32
10	LIVER	1:10	51.41	30.03	7.75
	KIDNEY	1:10	6.96	4.10	1.23
	INTESTINE	1:5	1.53	0.77	0.21
13	LIVER	1:10	5.20	3.06	0.89
	KIDNEY	1:10	12.44	7.32	2.06
	INTESTINE	1:5	15.04	7.52	1.71
17	LIVER	1:10	14.49	8.52	2.46
	KIDNEY	1:10	14.20	8.36	2.36
	INTESTINE	1:5	4.54	2.27	0.51
20	LIVER	1:10	7.25	4.26	0.86
	KIDNEY	1:10	10.16	5.98	1.74
	INTESTINE	1:5	3.00	1.50	0.39

[1] DAYS SUBSEQUENT TO ADDITION OF 500 PPM TUNGSTATE

* XANTHINE DEHYDROGENASE ACTIVITIES ARE RECORDED IN C. MM. OF O_2 CONSUMED PER 20 MINUTES.

ALL VALUES IN THIS TABLE REPRESENT DUPLICATE DETERMINATIONS FOR EACH TISSUE.

At a later date, 100 White Plymouth Rock pullets were treated as follows. Sixty of these pullets along with 15 males were placed on tungsten supplemented ration (500 ppm tungsten). Each male remained with the hens for three days and was removed and fed a molybdenum supplemented diet for six days. Forty hens were fed an unsupplemented ration and used as controls. After a 30-day depletion period, the eggs were collected by trap nest. They were then incubated and the chicks used for growth trials.

For simplicity the progeny from the hens fed the breeder-type ration shall be referred to as "non-supplemented chicks" and the progeny from the hens fed the breeder-type ration plus additional tungsten shall be referred to as "tungstate supplemented chicks". It should be pointed out that neither 250 ppm nor 500 ppm supplemental tungsten had any apparent effect on the rate of egg production or the per cent hatchability.

In order to determine the effect of tungsten supplementation on the gain of chicks and on the xanthin dehydrogenase levels, as measured manometrically, two chick trials of four-weeks duration were run. The rations used were the practical breeder-type rations, on which the dams were fed, as shown in Table IV. Xanthine dehydrogenase determinations were made from random samples of the "tungstate supplemented" and "non-supplemented" chicks at the beginning and at the end of the four-week trials in the manner previously described, using the Warburg constant volume respirometer.

The chicks were grown in starter-type battery brooders equipped with raised wire screen floors. The birds were weighed at bi-weekly intervals and individual gain and feed conversion data were collected.

All chicks were furnished feed and water ad libitum. The first trial involved 10 lots of eight chicks each.

As previously mentioned the "tungsten-supplemented chicks" and "non-supplemented chicks" were tested on both the practical breeder ration and the breeder ration plus 500 ppm supplemental tungsten. In this way the ration treatment as well as the dam treatment was evaluated. The four-week gain and feed conversion data are shown in Table VII.

TABLE VII

Results of the Study Concerning the Effect of Tungsten Supplementation on Gain, Feed Conversion, and Dam Treatment of Four-Week-Old Chicks Fed A Breeder Type Ration

Dam Treatment	Breeder Ration	Breeder Ration + Tungsten
	(Grams)	(Grams)
Non-supplemented	389.7(2.23)*	245.8(3.12)
Tungsten Supplemented	355.5(2.48)	258.2(2.99)

*Figures in parenthesis indicate feed conversion.

A statistical analysis of variance of the data obtained in this trial is shown in Table VIII. It was found that the tungsten supplementation caused a highly significant decrease in the rate of gain for the four-week-old birds from both dam treatments. The analysis of variance also revealed that the chicks from the dams fed the tungsten supplemented breeder ration grew at a significantly slower rate than did the chicks from dams fed the unsupplemented breeder ration. The

TABLE VIII

ANALYSIS OF VARIANCE SHOWING EFFECT OF 500 PPM TUNGSTEN ON RATE OF GAIN ON FOUR-WEEK OLD CHICKS ON BREEDER TYPE RATION

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	79	-	-
BETWEEN TREATMENTS	3	101.4	60.10**
NON-SUPPLEMENTED VS SUPPLEMENTED EFFECT OF RATION ON GROWTH	1	290.8	172.66*
NON-SUPPLEMENTED VS SUPPLEMENTED EFFECT OF DAM ON UNSUPPLEMENTED DIET	1	11.7	6.97*
NON-SUPPLEMENTED VS SUPPLEMENTED EFFECT OF DAM ON TUNGSTEN DIET	1	2.4	1.41
ERROR	76	1.7	-

*SIGNIFICANT AT 0.05 LEVEL OF PROBABILITY.

**SIGNIFICANT AT 0.01 LEVEL OF PROBABILITY.

RATION TREATMENT OF THE DAMS DID NOT APPEAR TO AFFECT THE RATE OF GAIN OF THE CHICKS FED THE TUNGSTEN SUPPLEMENTED RATION BUT THESE GAINS WERE INFERIOR TO THOSE EXPERIENCED ON THE UNSUPPLEMENTED RATION.

AN ANALYSIS OF VARIANCE WAS MADE ON THE FEED CONVERSION DATA OF THIS TRIAL. THIS ANALYSIS IS PRESENTED IN TABLE IX.

THERE WAS A SIGNIFICANT DEPRESSION OF FEED CONVERSION WHEN THE TUNGSTEN WAS FED TO THE CHICKS. THERE WAS ALSO A SIGNIFICANT DAM EFFECT EXHIBITED ON THE FEED CONVERSION ON THE FOUR-WEEK-OLD CHICKS. IN THE CASE OF BOTH THE UNSUPPLEMENTED AND TUNGSTEN SUPPLEMENTED RATIONS, IT WAS FOUND THAT THE SUPPLEMENTAL LEVEL OF TUNGSTEN FED TO

TABLE IX

ANALYSIS OF VARIANCE SHOWING EFFECT OF THE SUPPLEMENTATION OF 500 PPM TUNGSTEN ON FEED CONVERSION OF FOUR-WEEK-OLD CHICKS FED A BREEDER TYPE RATION

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	7	-	-
BETWEEN TREATMENTS	3	38.15	48.59*
NON-SUPPLEMENTED VS SUPPLEMENTED EFFECT OF RATION ON FEED CONVERSION	1	50.50	64.33*
NON-SUPPLEMENTED VS SUPPLEMENTED EFFECT OF DAM ON UNSUPPLEMENTED DIET	1	50.41	64.21*
NON-SUPPLEMENTED VS SUPPLEMENTED EFFECT OF DAM ON TUNGSTEN DIET	1	62.41	79.50*
ERROR	4	0.79	-

*SIGNIFICANT AT 0.05 LEVEL OF PROBABILITY.

THE HENS RESULTED IN A STATISTICALLY SIGNIFICANT DEPRESSION IN FEED CONVERSION OF CHICKS FED BOTH RATIONS.

AT THE TIME OF HATCHING, XANTHINE DEHYDROGENASE ACTIVITY WAS MEASURED ON 13 CHICKS FROM TUNGSTEN SUPPLEMENTED DAMS AND ON FIVE CHICKS FROM NON-SUPPLEMENTAL FED DAMS. THESE DETERMINATIONS ARE SHOWN IN TABLE X. AN ANALYSIS OF VARIANCE OF THESE DATA WAS MADE ON THE LEVELS OF XANTHINE DEHYDROGENASE LEVEL PER 100 MILLIGRAMS OF FRESH TISSUE. THIS ANALYSIS IS SHOWN IN TABLE XI. IT WAS FOUND THAT THE ENZYME LEVEL IN THE INTESTINE WAS HIGHLY SIGNIFICANTLY GREATER THAN THE XANTHINE DEHYDROGENASE LEVEL IN THE LIVER. THE ANALYSIS FURTHER

TABLE X

XANTHINE DEHYDROGENASE ACTIVITY OF VARIOUS TISSUES OF DAY-OLD CHICKS FROM DIFFERENT DAM TREATMENTS

DAM TREATMENT	TISSUE	XANTHINE DEHYDROGENASE ACTIVITY* PER FLASK	PER 100 MG TISSUE	PER MG N IN SAMPLE
TUNGSTEN SUPPLEMENTED	LIVER	14.41 ± 6.1	8.48 ± 3.6	2.55 ± 1.2
TUNGSTEN SUPPLEMENTED	INTESTINE	17.65 ± 6.7	9.26 ± 3.6	4.45 ± 1.1
NON-SUPPLEMENTED	LIVER	5.59 ± 0.9	3.29 ± 0.9	1.20 ± 0.01
NON-SUPPLEMENTED	INTESTINE	5.76 ± 1.5	3.98 ± 0.9	0.91 ± 0.01

*XANTHINE DEHYDROGENASE ACTIVITY MEASURED IN C. MM. OF O_2 CONSUMED PER 20 MINUTES.

TABLE XI

ANALYSIS OF VARIANCE OF XANTHINE DEHYDROGENASE LEVELS OF DAY-OLD-CHICKS HAVING DIFFERENT DAM TREATMENT

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	35	-	-
BETWEEN TREATMENTS	3	67.60	3.99*
LIVER VS INTESTINE	1	205.61	12.13**
LIVER: TUNGSTATE VS NON-TUNGSTATE SUPPLEMENTED	1	97.03	5.23*
INTESTINE: TUNGSTATE VS NON-TUNGSTATE SUPPLEMENTED	1	100.63	5.94*
ERROR	32	16.95	-

*SIGNIFICANT AT 0.05 LEVEL OF PROBABILITY.
**SIGNIFICANT AT 0.01 LEVEL OF PROBABILITY.

REVEALED THAT THE DAY-OLD CHICKS FROM THE TUNGSTATE FED DAMS HAD STATISTICALLY HIGHER LEVELS OF XANTHINE DEHYDROGENASE ACTIVITY THAN THAT OF THE CHICKS FROM NON-SUPPLEMENTED DAMS BOTH IN THE LIVER AND INTESTINAL TISSUES.

AT THE END OF THE FOUR-WEEK TRIAL, SAMPLES OF LIVER AND INTESTINE WERE TAKEN FROM CHICKS ON EACH TREATMENT AND THE XANTHINE DEHYDROGENASE LEVELS WERE DETERMINED. THE RESULTS OF THE DETERMINATIONS ARE SHOWN IN TABLE XII. AN ANALYSIS OF VARIANCE WAS MADE ON THE DATA PRESENTED IN TABLE XII AND REVEALED THAT THERE WERE NO REAL DIFFERENCES EXISTING IN THESE DATA. THE FAILURE OF THESE DIFFERENCES TO BE SIGNIFICANT PROBABLY IS DUE TO THE UNUSUALLY LARGE VARIATION OF THE VALUES FOR EACH TREATMENT.

ANOTHER TRIAL WAS INITIATED TO TEST THE EFFECT OF THE TUNGSTEN SUPPLEMENTATION ON A PRACTICAL-TYPE BROILER TYPE RATION (TABLE I).

TABLE XII

XANTHINE DEHYDROGENASE LEVELS OF CHICK TISSUES AT FOUR WEEKS OF AGE

DAM TREATMENT	BREEDER RATION		BREEDER RATION + TUNGSTEN	
	LIVER*	INTESTINE	LIVER	INTESTINE
NON-SUPPLEMENTED	5.02	4.82	5.20	5.43
TUNGSTEN SUPPLEMENTED	2.61	0.33	3.22	1.01

*XANTHINE DEHYDROGENASE ACTIVITIES ARE MEASURED IN C. MM. OF O_2 CONSUMED PER 20 MINUTES PER 100 MG. FRESH TISSUE.

THE SAME SUPPLEMENTAL LEVELS OF TUNGSTEN WERE USED IN THIS TRIAL AS IN THE PREVIOUS ONE. THERE WERE NO ENZYME DETERMINATIONS MADE DURING THIS CHICK GROWTH TRIAL. THE GAIN AND FEED CONVERSION DATA ARE PRESENTED IN TABLE XIII. THIS STUDY INVOLVED 10 LOTS OF 10 CHICKS EACH.

AN ANALYSIS OF VARIANCE WAS MADE ON THE 24-DAY GAIN OF THESE BIRDS. IT WAS FOUND, AS WITH THE BREEDER RATION, THAT THE 500 PPM SUPPLEMENTAL TUNGSTEN RESULTED IN A HIGHLY SIGNIFICANT DEPRESSION IN GAIN OF CHICKS FROM BOTH DAM TREATMENTS. IT WAS ALSO SHOWN THAT THE CHICKS FROM TUNGSTEN-SUPPLEMENTED DAMS EXHIBITED A SIGNIFICANT DEPRESSION IN THE GROWTH RATE WHEN FED THE TUNGSTEN-SUPPLEMENTED BROILER RATION. A HIGHLY SIGNIFICANT DEPRESSION IN THE GROWTH RATE OF THESE SAME CHICKS WAS OBSERVED WHEN THEY WERE FED NON-SUPPLEMENTED BROILER RATION. THIS ANALYSIS OF VARIANCE IS SHOWN IN TABLE XIV.

TABLE XIII

RESULTS OF TWENTY-FOUR-DAY-OLD CHICKS SHOWING EFFECT OF 500 PPM TUNGSTEN ON GAIN, FEED CONVERSION AND DAM TREATMENT (BROILER RATION)

DAM TREATMENT	PRACTICAL BROILER RATION	PRACTICAL BROILER RATION + TUNGSTEN
	(GRAMS)	(GRAMS)
NON-SUPPLSMENTED	404(1.74)*	204(2.78)
TUNGSTEN SUPPLEMENTED	367(1.96)	185(2.44)

*FIGURES IN PARENTHESIS INDICATE FEED CONVERSION.

TABLE XIV

ANALYSIS OF VARIANCE SHOWING EFFECT OF 500 PPM TUNGSTEN ON RATE OF GAIN OF CHICKS FED A BROILER RATION

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	99		
BETWEEN TREATMENTS	3	31.07	16.69**
NORMAL VS TUNGSTEN RATION	1	49.42	26.50**
TUNGSTEN VS NON-SUPPLEMENTED EFFECT OF DAM ON NORMAL RATION	1	16.16	8.68**
TUNGSTEN VS NON-SUPPLEMENTED EFFECT OF DAM ON TUNGSTEN RATION	1	5.02	2.70
ERROR	96	1.78	

** SIGNIFICANT AT 0.01 LEVEL OF PROBABILITY

TABLE XV

ANALYSIS OF VARIANCE SHOWING EFFECT OF 500 PPM TUNGSTEN ON FEED CONVERSION OF CHICKS ON A BROILER RATION

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	7		
BETWEEN TREATMENTS	3	44.52	56.35*
TUNGSTEN VS NORMAL DIET	1	117.04	148.15**
EFFECT OF DAM ON NORMAL DIET	1	4.63	5.86*
EFFECT OF DAM ON TUNGSTEN DIET	1	1.19	1.50
ERROR	4	0.79	

* SIGNIFICANT AT 0.05 LEVEL OF PROBABILITY

** SIGNIFICANT AT 0.01 LEVEL OF PROBABILITY

THE FEED CONVERSION DATA WERE ANALYZED BY AN ANALYSIS OF VARIANCE. (TABLE XV) A HIGHLY SIGNIFICANT DEPRESSION IN FEED CONVERSION WAS CAUSED BY THE TUNGSTEN SUPPLEMENTATION OF THE PRACTICAL BROILER RATION. THERE APPEARED TO BE A DIFFERENCE DUE TO THE TREATMENT OF THE DAMS BUT THIS DID NOT PROVE TO BE STATISTICALLY SIGNIFICANT.

SUMMARY

THE ADDITION OF 250 PPM TUNGSTEN TO THE BREEDER RATION APPEARED TO HAVE LITTLE OR NO EFFECT ON THE XANTHINE DEDYDROGENASE ACTIVITY OF THE HEN TISSUES TESTED. THE SUPPLEMENTATION OF 500 PPM TUNGSTEN CAUSED A STEADY DECLINE IN THIS ENZYME ACTIVITY WHICH APPEARED TO TAPER OFF TO A LOW LEVEL AFTER A PERIOD OF 30 DAYS. NEITHER OF THESE LEVELS OF ADDED TUNGSTEN HAD AN EFFECT ON THE RATE OF EGG PRODUCTION OR HATCHABILITY.

IT WAS FOUND THAT DAY-OLD-CHICKS, FROM DAMS FED THE TUNGSTATE SUPPLEMENTED RATION, HAD SIGNIFICANTLY HIGHER AMOUNTS OF XANTHINE DEHYDROGENASE IN THE LIVER AND INTESTINE THAN DID CHICKS FROM DAMS FED THE NON-SUPPLEMENTED RATION. CHICKS FROM DAMS FED THE TUNGSTEN SUPPLEMENTED RATION GREW AT A SIGNIFICANTLY LOWER RATE THAN DID THE CONTROL CHICKS ON BOTH THE BREEDER RATION AND THE BROILER RATION. WHEN THE CHICKS RATION WAS SUPPLEMENTED WITH 500 PPM TUNGSTEN, A SLOWER RATE OF GAIN WAS OBSERVED WHEN COMPARED TO RATIONS CONTAINING NO SUPPLEMENTATION.

AT FOUR-WEEKS OF AGE, THERE APPEARED TO BE NO DIFFERENCE IN THE XANTHINE DEHYDROGENASE ACTIVITIES BETWEEN THE CHICKS REGARDLESS OF THE RATION FED OR THE DAM TREATMENT. THE VARIATION THAT OCCURRED IN THE

ENZYME DETERMINATIONS WAS LARGE AND THE SAMPLE SIZE TOO SMALL AND THIS MIGHT HAVE ACCOUNTED FOR THERE BEING NO STATISTICALLY SIGNIFICANT DIFFERENCES BETWEEN TREATMENTS.

C. Molybdate Supplementation of Chick Diets

Since it appeared that tungstate supplementation of the breeder hens' diet produced chicks deficient in molybdenum, it was decided to use these chicks in evaluating the effect of adding various levels of molybdenum to the chicks' diet. The anomalous response of the chicks from tungstate supplemented breeder hens and from non-supplemented breeder hens raised some question as to the nature of the condition produced by the tungstate supplementation. It was decided, therefore, to test the various additions of molybdenum to the rations of chicks from both tungstate supplemented breeders and non-supplemented breeders.

The purpose of this study was to test the effect of molybdenum supplementation at various levels of a purified diet and a practical corn-soybean ration.

A purified cerelose-Drackett protein ration was formulated and was calculated to contain less one ppm in molybdenum. This purified diet is shown in Table XVI. Molybdenum supplementations of 0, 1, 10, 50 and 100 ppm were added to this basal ration and these rations were fed to replicate lots of 10 chicks each on each ration.

It was found that this ration did not support sufficient chick growth to competently test the additives. Statistical analysis of the results of these trials revealed that there were no real differences, probably due to the large variation which occurred within each group.

Another purified cerelose-Drackett protein diet was formulated. The protein content was increased eight per cent at the expense of cerelose. A higher energy ration was attained by increasing the Wesson Oil content by one per cent. A one per cent level of methionine

TABLE XVI

PURIFIED CHICK TEST RATION

INGREDIENT	PER CENT
CERELOSE	59.00
DRACKETT PROTEIN	22.00
JONES-FOSTER SALT MIX #12	4.30
CELLULOSE	4.00
CRISCO	4.00
WESSON OIL	2.00
COD LIVER OIL	1.00
GLYCINE	2.10
L-ARGININE	1.01
L-CYSTINE	0.06

THE FOLLOWING MATERIALS WERE ADDED AS GRAMS PER POUND:

	GM/LB		GM/LB
ALPHA TOCOPHEROL	0.005	RIBOFLAVIN	0.003
CHOLINE CHLORIDE	0.90	BIOTIN	0.00014
INOSITOL	0.45	FOLIC ACID	0.002
PARA-AMINOBENZOIC ACID	0.02	THIAMIN HCL	0.0014
NIACIN	0.01	VITAMIN B_{12}	0.00001
CALCIUM PANTOTHENATE	0.01	MENADIONE	0.004
PYRIDOXINE	0.003		

WAS ADDED AT THE EXPENSE OF GLYCINE. CREATINE HYDRATE WAS ADDED AT THE LEVEL OF 0.25 PER CENT AND THE LEVELS OF RIBOFLAVIN AND BIOTIN WERE DOUBLED. DIPHENYL-P-PHENELEDIAMINE (DPPD) WAS ADDED AS AN ANTI-OXIDANT. THIS PURIFIED RATION IS PRESENTED IN TABLE XVII. THE CALCULATED AMINO ACID COMPOSITION OF THIS RATION IS SHOWN IN TABLE XVIII AND IT WILL BE NOTED THAT THE REQUIREMENT FOR EACH AMINO ACID IS MET, AS ESTABLISHED BY SCOTT ET AL (1957).

AFTER SUBSTANTIATING THE FACT THAT THIS RATION WOULD ADEQUATELY SUPPORT GROWTH, IT, AS WELL AS THE PRACTICAL CORN-SOYBEAN MEAL BROILER RATION (TABLE 1), WAS CHEMICALLY ANALYZED FOR MOLYBDENUM, TUNGSTEN, COPPER, SULFUR, PHOSPHORUS, AND POTASSIUM.

MOLYBDENUM WAS DETERMINED AS A DITHIOL (4-METHYL-1,2-DIMERCAPTO-BENZENE) COMPLEX DEVELOPED IN N-AMYL ACETATE. TUNGSTEN, FROM THE SAME SAMPLE, WAS REDUCED BY STANNOUS CHLORIDE AND TITANIUM III, AND WAS DETERMINED AS A TUNGSTEN-DITHIOL COMPOUND ALSO DEVELOPED IN N-AMYL ACETATE. THIS METHOD DEVELOPED BY TEEKELL AND ASSOCIATES IS FULLY DESCRIBED IN APPENDIX I. COPPER WAS DETERMINED BY A DIETHYLDITHIO-CARBAMATE METHOD AND SULFUR WAS DETERMINED BY A GRAVIMETRIC METHOD AS BARIUM SULFATE; THESE PROCEDURES WERE DESCRIBED BY PARKS ET AL (1943). PHOSPHORUS WAS DETERMINED PHOTOMETRICALLY USING VANADOMOLYBDATE (EPPS 1950, AND AUSTIN, DENSON, AND EPPS 1953). POTASSIUM WAS DETERMINED BY THE USE OF A BECKMAN FLAME SPECTROPHOTOMETER IN THE MANNER DESCRIBED BY AUSTIN AND ASSOCIATES (1953).

THE AMOUNTS FOUND IN THE PURIFIED RATION WERE: MOLYBDENUM, 2.0 PPM; TUNGSTEN, 0.2 PPM, COPPER, 25.9 PPM; SULFUR, 11649 PPM; PHOSPHORUS, 5988 PPM; AND POTASSIUM, 4296 PPM. THE PRACTICAL CORN-SOYBEAN MEAL BROILER RATION CONTAINED: MOLYBDENUM, 1.5 PPM; TUNGSTEN, 0.4 PPM;

TABLE XVII

PURIFIED CHICK RATION

INGREDIENT	PER CENT
CERELOSE	50.40
DRACKETT PROTEIN	30.00
JONES-FOSTER SALT MIX #12	4.30
CELLULOSE	4.00
CRISCO	4.00
WESSON OIL	3.00
COD LIVER OIL	1.00
GLYCINE	1.00
METHIONINE	1.00
CREATINE HYDRATE	0.25

THE FOLLOWING MATERIALS WERE ADDED AS GRAMS PER POUND:

	GM/LB		GM/LB
ALPHA TOCOPHEROL	0.05	RIBOFLAVIN	0.007
CHOLINE CHLORIDE	0.90	BIOTIN	0.0027
INOSITOL	0.45	FOLIC ACID	0.002
PARA-AMINOBENZOIC ACID	0.02	THIAMINE HCL	0.0014
CALCIUM PANTOTHENATE	0.01	MENADIONE	0.004
NIACIN	0.01	VITAMIN B_{12}	0.00001
PYRIDOXINE	0.003	DPPD	0.01
VITAMIN D_3	0.00375		

TABLE XVIII

AMINO ACID COMPOSITION OF PURIFIED RATION SHOWN IN TABLE XVII

INGREDIENT	CONTENT (PER CENT)	REQUIREMENT[1] (PER CENT)
ARGININE	2.49	1.40
HISTIDINE	0.78	0.51
ISOLEUCINE	1.95	0.84
LEUCINE	2.25	2.26
LYSINE	2.04	1.54
METHIONINE	1.33[2]	0.45 OR 0.2
CYSTINE	0.18	0 OR 0.4
PHENYLALANINE	1.50	0.59 OR 0.44
TYROSINE	1.02	0.7 OR 0.8
THREONINE	1.17	0.58
TRYPTOPHAN	0.33	0.175
VALINE	1.65	0.96
ALANINE	1.08	-
ASPARTIC ACID	1.86	-
GLUTAMIC ACID	5.85	15.00
GLYCINE	2.08[2]	0.5
PROLINE	0.75	-
SERINE	2.07	-

[1] AMINO ACID REQUIREMENTS AS ESTABLISHED BY SCOTT ET AL (1957).

[2] ADDED: 1% METHIONINE
1% GLYCINE
0.25% CREATINE HYDRATE

COPPER, 10.8 PPM; SULFUR, 4635 PPM; PHSOPHORUS, 3419 PPM; AND POTASSIUM, 9350 PPM.

MOLYBDENUM AND TUNGSTEN DETERMINATIONS WERE ALSO MADE ON WHOLE CHICK CARCASSES, LIVERS, AND KIDNEYS (WET WEIGHT BASES) OF DAY-OLD CHICKS FROM BOTH TUNGSTEN-SUPPLEMENTED AND NON-SUPPLEMENTED BREEDER HENS. THIS WAS DONE TO DETERMINE THE CARRY-OVER OF THESE ELEMENTS FROM THE DAM. THESE RESULTS ARE SHOWN IN TABLE XIX. FIVE DETERMINATIONS WERE MADE FROM EACH SAMPLE.

TABLE XIX

MOLYBDENUM AND TUNGSTEN LEVELS FOUND IN DAY-OLD CHICKS FROM DIFFERENT DAM TREATMENT

DAM TREATMENT	TISSUE	MOLYBDENUM (PPM)	TUNGSTEN (PPM)
NON-SUPPLEMENTED	WHOLE CARCASS	0.19	0.23
NON-SUPPLEMENTED	LIVER	1.14	0.79
NON-SUPPLEMENTED	KIDNEY	4.71	2.75
TUNGSTEN SUPPLEMENTED	WHOLE CARCASS	0.19	0.33
TUNGSTEN SUPPLEMENTED	LIVER	1.19	1.78
TUNGSTEN SUPPLEMENTED	KIDNEY	5.26	7.21

ATTENTION IS CALLED TO THE FACT THAT THERE WAS A CARRY-OVER OF THE TUNGSTEN FROM THE DAMS TO THE CHICKS. THIS IS PARTICULARLY EVIDENT IN THE CASE OF KIDNEY AND LIVER.

FOR CLARITY THE FEEDING TRIALS WITH CHICKS FROM EACH DAM TREATMENT AND EACH RATION TREATMENT WILL BE DESCRIBED INDIVIDUALLY. IT IS

BELIEVED THAT SUCH A DESCRIPTION MAY AVOID SOME MISUNDERSTANDING.

1. Purified Ration Studies with Chicks From Tungstate Supplemented Hens

ALL CHICKS WERE RAISED IN STARTER TYPE BATTERY BROODERS EQUIPPED WITH RAISED WIRE SCREEN FLOORS. FEED AND WATER WERE SUPPLIED *AD LIBITUM*. LEVELS OF MOLYBDENUM OF 0, 1, 2, 3, 5, 10, 50, 100, 500, 1000, AND 2000 PPM WERE TESTED. THIS STUDY INVOLVED 936 BIRDS IN A SERIES OF 10 TRIALS. WITHIN EACH TRIAL EACH MOLYBDENUM LEVEL WAS FED TO REPLICATE LOTS OF 10 CHICKS EACH.

THE 20-DAY GAIN AND FEED CONVERSION DATA FROM THIS STUDY ARE PRESENTED IN TABLE XX. AN ANALYSIS OF VARIANCE WAS MADE ON THE RATE OF GAIN IN THIS STUDY. THIS ANALYSIS IS SHOWN IN TABLE XXI.

THIS ANALYSIS INDICATED THAT THE ADDITION OF 1, 10, AND 50 PPM OF MOLYBDENUM GAVE A HIGHLY SIGNIFICANT IMPROVEMENT IN THE RATE OF GAIN, WHILE THE ADDITION OF 2, 3, 5 AND 100 PPM SUPPLEMENTAL MOLYBDENUM GAVE A STATISTICALLY SIGNIFICANT INCREASE IN THE RATE OF GAIN FOR THESE CHICKS. THE MOLYBDENUM TOXICITY LEVEL APPARENTLY LIES BETWEEN 100 AND 500 PPM AS THE ADDITION OF 500 PPM MOLYBDENUM RESULTED IN A HIGHLY SIGNIFICANT GROWTH DEPRESSION. HIGHER LEVELS OF MOLYBDENUM SUPPLEMENTATION (1000 AND 2000 PPM) GAVE EVEN GREATER REDUCTION IN GAIN WHICH WERE HIGHLY SIGNIFICANT.

AN ANALYSIS OF VARIANCE WAS MADE ON THE FEED CONVERSION DATA FROM THIS TRIAL AND IS PRESENTED IN TABLE XXII. IT WAS REVEALED FROM THIS ANALYSIS THAT THE LOWER LEVELS OF MOLYBDENUM SUPPLEMENTATION, *I.E.* ONE, TWO AND THREE PPM, HAD NO SIGNIFICANT EFFECT ON FEED CONVERSION IN THESE 20-DAY TRIALS. THE ADDITION OF FIVE PPM MOLYBDENUM GAVE A SIGNIFICANT IMPROVEMENT IN THE FEED CONVERSION, AND THE SUPPLEMENTATION OF 10 PPM

TABLE XX

GAIN AND FEED CONVERSION DATA ON CHICKS, FROM TUNGSTATE SUPPLEMENTED DAMS, FED A PURIFIED DIET WITH MOLYBDENUM SUPPLEMENTATION

ADDED MOLYBDENUM (PPM)	GAIN (GRAMS)	FEED CONVERSION
0	169	1.50
1	179	1.47
2	177	1.47
3	176	1.47
5	179	1.41
10	191	1.33
50	190	1.41
100	178	1.42
500	153	1.56
1000	135	1.85
2000	83	1.91

TABLE XXI

ANALYSIS OF VARIANCE OF 20-DAY GAIN OF CHICKS, FROM TUNGSTATE SUPPLEMENTED DAMS, FED A PURIFIED RATION PLUS VARIOUS LEVELS OF MOLYBDENUM

SOURCE	DEGREES OF FREEDOM	MEAN SQAURE	F VALUE
TOTAL	935		
BETWEEN MOLYBDENUM LEVELS	10	20364.0	53.90**
0 VS 1 PPM MOLYBDENUM	1	4494.0	11.91**
0 VS 2 PPM MOLYBDENUM	1	2246.0	5.14*
0 VS 3 PPM MOLYBDENUM	1	2142.0	5.65*
0 VS 5 PPM MOLYBDENUM	1	2178.0	5.79*
0 VS 10 PPM MOLYBDENUM	1	22731.1	60.13**
0 VS 50 PPM MOLYBDENUM	1	21374.0	56.51**
0 VS 100 PPM MOLYBDENUM	1	1690.0	4.47*
0 VS 500 PPM MOLYBDENUM	1	3725.0	9.85**
0 VS 1000 PPM MOLYBDENUM	1	15977.0	42.27**
0 VS 2000 PPM MOLYBDENUM	1	96683.0	255.70**
ERROR	925	378.0	-

* SIGNIFICANT AT 0.05 LEVEL OF PROBABILITY

** SIGNIFICANT AT 0.01 LEVEL OF PROBABILITY

TABLE XXII

ANALYSIS OF VARIANCE OF FEED CONVERSION DATA ON CHICKS, FROM TUNGSTATE SUPPLEMENTED DAMS, FED A PURIFIED RATION PLUS MOLYBDENUM SUPPLEMENTS

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	78		
BETWEEN MOLYBDENUM LEVELS	10	893	7.03**
0 VS 1 PPM MOLYBDENUM	1	102	0.80
0 VS 2 PPM MOLYBDENUM	1	54	0.42
0 VS 3 PPM MOLYBDENUM	1	76	0.60
0 VS 5 PPM MOLYBDENUM	1	595	4.68*
0 VS 10 PPM MOLYBDENUM	1	1016	8.01**
0 VS 50 PPM MOLYBDENUM	1	545	4.29*
0 VS 100 PPM MOLYBDENUM	1	547	4.30*
0 VS 500 PPM MOLYBDENUM	1	198	1.56
0 VS 1000 PPM MOLYBDENUM	1	2140	16.55**
0 VS 2000 PPM MOLYBDENUM	1	2872	22.61**
ERROR	68	127	

* SIGNIFICANT AT 0.05 LEVEL OF PROBABILITY

** SIGNIFICANT AT 0.01 LEVEL OF PROBABILITY

GAVE A HIGHLY SIGNIFICANT IMPROVEMENT IN FEED CONVERSION. AT SOMEWHAT HIGHER LEVELS OF SUPPLEMENTATION (50 AND 100 PPM) THE IMPROVED FEED CONVERSION WAS NOT AS GREAT AS IN THE CASE OF 10 PPM LEVEL OF SUPPLEMENTATION, BUT WAS STILL A STATISTICALLY SIGNIFICANT IMPROVEMENT IN THE UTILIZATION OF FEED WHEN COMPARED TO THE NON-MOLYBDENUM SUPPLEMENTED RATION. THE ADDITION OF 500 PPM MOLYBDENUM RESULTED IN THE SAME FEED CONVERSION AS WAS OBTAINED ON THE BASAL RATION. HIGHER LEVELS OF SUPPLEMENTATION, THAT IS 1000 AND 2000 PPM, GAVE A HIGHLY SIGNIFICANT DEPRESSION OF FEED CONVERSION. IT IS BELIEVED THAT THESE HIGHER LEVELS OF SUPPLEMENTATION WERE DEFINITELY TOXIC TO THE CHICKS, ALTHOUGH NO GROSS CLINICAL SYMPTOMS OR LESIONS WERE OBSERVED.

IN THE WORK REPORTED IN THE PREVIOUS SECTION ON XANTHINE DEHYDROGENASE, AS MEASURED BY THE WARBURG CONSTANT VOLUME RESPIROMETER, CONSIDERABLE VARIATION WAS ENCOUNTED IN THE LEVELS OF THIS ENZYME. IN AN ATTEMPT TO SECURE LESS VARIABLE VALUES FOR THIS ENZYME, IT WAS DECIDED TO MEASURE URIC ACID SYNTHESIS BY LIVER AND KIDNEY TISSUES FOR USE AS AN INDICATOR OF XANTHINE DEHYDROGENASE ACTIVITY. THE METHOD FOR THIS DETERMINATION IS GIVEN IN APPENDIX II. TWO CHICKS FROM THE GROUP FED 3, 5, 50, 100, AND 2000 PPM OF SUPPLEMENTAL MOLYBDENUM WERE KILLED AND URIC ACID SYNTHESIS WAS DETERMINED IN LIVER AND KIDNEY TISSUES. THE RESULTS OF THESE ANALYSES ARE SHOWN IN TABLE XXIII. AS MAY BE SEEN FROM THESE RESULTS, THE ADDED LEVELS OF MOLYBDENUM APPEARED TO HAVE NO EFFECT ON URIC ACID SYNTHESIS IN EITHER THE LIVER OR KIDNEY.

IT WAS THEN DECIDED TO MAKE OTHER PHYSIOLOGICAL TESTS. A DIFFERENCE IN ALKALINE PHOSPHOSPHATASE ACTIVITY HAD BEEN OBSERVED (CANTAROW AND TRUMPER, 1955) DURING CERTAIN HEPHATIC AND RENAL MALFUNCTIONS. SINCE

TABLE XXIII

URIC ACID DETERMINATIONS ON VARIOUS TISSUES OF CHICKS, FROM TUNGSTATE-FED DAMS, FED PURIFIED RATION PLUS MOLYBDENUM SUPPLEMENTATION

ADDED MOLYBDENUM (PPM)	20-DAY GAIN (GRAMS)	10 MINUTE ENDOGENOUS OXIDATION	30 MINUTE ENDOGENOUS OXIDATION	NET 20 MINUTE ENZYMATIC OXIDATION#
		LIVER*		
0	230	0.26	0.29	5.28
3	237	0.60	0.64	5.96
5	239	0.45	0.46	5.86
50	245	0.37	0.46	6.08
100	232	0.28	0.34	4.85
2000	125	0.21	0.42	5.92
		KIDNEY*		
0	230	0.50	0.55	6.50
3	237	0.56	0.75	6.18
5	239	0.58	0.71	4.59
50	245	0.56	0.69	5.24
100	232	0.51	0.62	6.53
2000	125	0.52	0.56	6.27

*URIC ACID EXPRESSED AS MILLIGRAMS URIC ACID PER GRAM TISSUE.

#NET ENZYMATIC OXIDATION = GROSS ENZYMATIC OXIDATION MINUS (30 MINUTE PLUS 10 MINUTE) ENDOGENOUS OXIDATION.

HIGH LEVELS OF TUNGSTEN WERE FOUND TO BE CONTAINED IN KIDNEYS OF CHICKS FROM TUNGSTATE SUPPLEMENTED DAMS, IT WAS DECIDED TO DETERMINE CORRELATIONS BETWEEN THE ACTIVITY OF THIS ENZYME AND KIDNEY TUNGSTEN LEVEL.

ALKALINE PHOSPHATASE LEVELS WERE DETERMINED BY THE METHOD DESCRIBED IN APPENDIX III.

CHICKS WERE SELECTED FROM THE VARIOUS GROUPS, SLAUGHTERED AND ALKALINE PHOSPHATASE DETERMINATIONS MADE ON LIVER AND KIDNEY TISSUES. THESE VALUES ARE FOUND IN TABLE XXIV. IT SHOULD BE POINTED OUT THAT, WHILE THERE VALUES MAY NOT BE ABSOLUTE, THEY CERTAINLY ARE RELATIVE SINCE ALL DETERMINATIONS WERE MADE AT THE SAME TIME. GOOD AGREEMENT WAS NOTED BETWEEN REPLICATE DETERMINATIONS. THERE DID NOT APPEAR TO BE ANY DEFINITE TREND IN THE LIVER VALUES UNTIL THE SUSPECTED TOXIC LEVELS WERE REACHED. THE LIVER VALUES FOR THESE LEVELS (500, 1000, 2000, AND 3000 PPM MOLYBDENUM) WERE FROM 1.5 TO 2.0 TIMES THE VALUES OBTAINED WITH THE UNSUPPLEMENTED CHICKS. THE VALUES FOR THE KIDNEY TISSUE APPEAR TO FOLLOW A MORE DEFINITE TREND AS THE LEVEL OF MOLYBDENUM WAS INCREASED.

SINCE HIGHER ALKALINE PHOSPHATASE LEVELS MAY BE ASSOCIATED WITH CERTAIN BONE MALFUNCTIONS, IT WAS DECIDED TO DETERMINE BONE ASH IN THE MANNER PRESCRIBED BY A. O. A. C. TWO CHICKS EACH FROM GROUPS FED THREE LEVELS OF MOLYBDENUM WERE SELECTED, SLAUGHTERED AND BONE ASH WAS DETERMINED ON THE LEFT TIBIA. AS MAY BE SEEN FROM THE RESULTS (TABLE XXV), A STATISTICAL ANALYSIS OF VARIANCE DID NOT REVEAL ANY REAL DIFFERENCES IN THE PER CENT BONE ASH OF THE 20-DAY OLD CHICKS. MOLYBDENUM DID NOT APPEAR TO AFFECT BONE FORMATION IN THESE GROWING CHICKS.

II. Purified Ration Studies With Chicks From Non-Supplemented Dams

THE PROCEDURES FOLLOWED HERE WERE THE SAME AS THOSE DESCRIBED IN SECTION I. THE CHICKS WERE FROM WHITE PLYMOUTH ROCK HENS FED THE NON-SUPPLEMENTED PRACTICAL BREEDER RATION. THE PURPOSE OF THESE TRIALS

TABLE XXIV

ALKALINE PHOSPHATASE LEVELS ON VARIOUS TISSUES OF CHICKS, FROM TUNGSTATE DAMS, FED PURIFIED RATION PLUS MOLYBDENUM SUPPLEMENTATION

ADDED MOLYBDENUM (PPM)	CONTROL (MG. P*)	INCUBATED (MG. P*)	NET YIELD (MG. P*)
	LIVER		
0	12.16	65.59	53.43
2	13.24	64.11	50.88
3	10.85	65.80	54.96
5	10.39	67.49	57.02
50	10.57	63.27	52.70
100	5.27	70.99	62.72
500	9.19	82.68	73.49
1000	9.19	88.14	78.95
2000	8.92	92.63	83.71
3000	8.55	117.26	108.71
	KIDNEY		
0	10.43	45.78	36.35
2	10.11	46.40	36.29
3	8.36	51.15	42.78
5	7.35	52.68	45.32
50	9.01	62.43	53.42
100	10.57	64.11	53.54
500	9.93	65.46	55.53
1000	9.47	67.49	58.03
2000	9.10	79.30	70.20
3000	10.29	82.71	72.42

*VALUES EXPRESSED AS MILLIGRAMS PHOSPHORUS PER GRAM TISSUE (WET WEIGHT)

TABLE XXV

BONE ASH OF CHICKS, FROM TUNGSTATE SUPPLEMENTED DAMS, FED PURIFIED DIET PLUS SUPPLEMENTAL MOLYBDENUM

ADDED MOLYBDENUM (PPM)	PER CENT BONE ASH
2	50.91
2	50.99
500	50.33
500	51.29
3000	50.32
3000	50.72

WAS TO DETERMINE THE EFFECT OF THE FEEDING OF VARIOUS LEVELS OF MOLYBDENUM IN A PURIFIED DIET TO CHICKS WHOSE DAMS HAD NOT RECEIVED A METABOLIC ANTAGONIST. THE SUPPLEMENTAL LEVELS OF MOLYBDENUM WERE THE SAME AS USED IN THE PREVIOUS SECTION.

THE 20-DAY GAINS AND FEED CONVERSIONS ARE SHOWN IN TABLE XXVI. AN ANALYSIS OF VARIANCE WAS MADE ON THE GROWTH RATE IN THIS SERIES. THIS ANALYSIS IS SHOWN IN TABLE XXVII. SUPPLEMENTAL LEVELS OF 1, 2, 3, 5, 10, 50, 100, AND 500 PPM MOLYBDENUM HAD NO REAL EFFECT ON GROWTH OF CHICKS FROM NON-SUPPLEMENTED DAMS. IT SHOULD BE NOTED THAT IN THE PREVIOUS STUDY THE ADDITION OF 500 PPM MOLYBDENUM GAVE A HIGHLY SIGNIFICANT DEPRESSION IN GROWTH AND SUGGESTED THAT THIS MIGHT BE A TOXIC LEVEL, BUT IN CHICKS, FROM NON-SUPPLEMENTED DAMS, THIS LEVEL OF MOLYBDENUM HAD NO REAL EFFECT. THE MOLYBDENUM TOXICITY LEVEL OF THESE CHICKS APPEARS TO BE CONSIDERABLY HIGHER, AS 1000 PPM

TABLE XXVI

GAIN AND FEED CONVERSIONS OF CHICKS, FROM NON-SUPPLEMENTED DAMS, FED PURIFIED DIET WITH MOLYBDENUM SUPPLEMENTATIONS

ADDED MOLYBDENUM (PPM)	GAIN (GRAMS)	FEED CONVERSIONS
0	177	1.52
1	177	1.47
2	171	1.46
3	172	1.49
5	173	1.46
10	183	1.47
50	182	1.47
100	182	1.49
500	175	1.53
1000	142	1.65
2000	99	1.76

SUPPLEMENTAL MOLYBDENUM ADDITION CAUSED A STATISTICALLY SIGNIFICANT DEPRESSION IN GROWTH RATE, BUT A HIGHLY SIGNIFICANT DEPRESSION IN GROWTH RATE WAS NOT REACHED UNTIL THE SUPPLEMENTAL LEVEL OF MOLYBDENUM WAS 2000 PPM.

IN ORDER TO DETERMINE IF THERE WAS A DAM EFFECT EXHIBITED IN THE CHICKS FED THE PURIFIED RATION, AN ANALYSIS OF VARIANCE WAS MADE COMPARING THE ENTIRE SERIES IN THE PREVIOUS SECTION WITH THE DATA OBTAINED IN THIS SERIES OF TRIALS. THIS ANALYSIS IS SHOWN IN TABLE XXVIII. IT WAS FOUND THAT THE NON-SUPPLEMENTED CHICKS GREW AT A

TABLE XXVII

ANALYSIS OF VARIANCE ON GAINS OF CHICKS, FROM NON-SUPPLEMENTED DAMS, FED PURIFIED RATION WITH MOLYBDENUM SUPPLEMENTATION

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	853	-	-
BETWEEN MOLYBDENUM LEVELS	10	28620	43.89**
0 VS 1 PPM MOLYBDENUM	1	12	0.01
0 VS 2 PPM MOLYBDENUM	1	1460	2.24
0 VS 3 PPM MOLYBDENUM	1	1395	2.14
0 VS 5 PPM MOLYBDENUM	1	1350	2.07
0 VS 10 PPM MOLYBDENUM	1	1506	2.31
0 VS 50 PPM MOLYBDENUM	1	1324	2.03
0 VS 100 PPM MOLYBDENUM	1	1291	1.98
0 VS 500 PPM MOLYBDENUM	1	125	0.20
0 VS 1000 PPM MOLYBDENUM	1	6247	9.58*
0 VS 2000 PPM MOLYBDENUM	1	177347	272.01**
ERROR	843	652	-

*SIGNIFICANT AT 0.05 LEVEL OF PROBABILITY
**SIGNIFICANT AT 0.01 LEVEL OF PROBABILITY

TABLE XXVIII

ANALYSIS OF VARIANCE COMPARING THE RATE OF GAIN BETWEEN CHICKS FROM SUPPLEMENTED AND NON-SUPPLEMENTED DAMS

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	1789	-	-
BETWEEN TREATMENTS	1	26569.9	51.2**
ERROR	1788	518.9	-

**SIGNIFICANT AT 0.01 LEVEL OF PROBABILITY

RATE WHICH WAS HIGHLY SIGNIFICANTLY SUPERIOR TO THE CHICKS FROM THE TUNGSTEN-SUPPLEMENTED DAMS. THIS SUGGESTS THAT THE TUNGSTEN FED TO THE PARENTS CAUSED A STATISTICALLY SIGNIFICANT DEPRESSION IN THE RATE OF GAIN FOR A PERIOD OF AT LEAST 20 DAYS. ALTHOUGH THESE DIFFERENCES BETWEEN DAM TREATMENTS WERE FOUND TO EXIST, ATTENTION IS CALLED TO THE FACT THAT THE CHICKS FROM THE TUNGSTATE SUPPLEMENTED DAMS, WHEN FED THE PURIFIED DIET CONTAINING 10 OR 50 PPM MOLYBDENUM, SHOWED A GREATER INCREASE IN THE RATE OF GAIN THAN DID CHICKS FROM NON-SUPPLEMENTED DAMS, WHEN SUPPLEMENTED WITH MOLYBDENUM AT ANY LEVEL. THIS SUGGESTS A SYNERGISTIC RELATIONSHIP OF TUNGSTEN AND MOLYBDENUM. THE FACT THAT THE NUMBER OF CHICKS IS RATHER LARGE (AN AVERAGE OF 20 GROUPS OF 10 CHICKS EACH) ADDS CONFIDENCE THAT THIS DIFFERENCE IN THE RATES OF GAIN MIGHT BE A REAL DIFFERENCE.

AN ANALYSIS OF VARIANCE WAS MADE ON THE FEED CONVERSION DATA IN THIS SERIES OF TRIALS. THIS ANALYSIS, PRESENTED IN TABLE XXIX, SHOWED THAT A STATISTICALLY SIGNIFICANT DIFFERENCE EXISTED IN THESE DATA. UPON SEPARATING THE VARIOUS COMPONENTS IT WAS FOUND THAT, AS IN THE CASE OF THE GAIN DATA, SUPPLEMENTAL LEVELS OF 1, 2, 3, 10, , 50, 100, AND 500 PPM MOLYBDENUM HAD NO SIGNIFICANT EFFECT ON THE FEED CONVERSIONS OF THE CHICKS FROM THE NON-SUPPLEMENTED DAMS. THE ADDITION OF 1000 PPM MOLYBDENUM CAUSED A SIGNIFICANT DEPRESSION AND THE INCLUSION OF 2000 PPM MOLYBDENUM CAUSED A HIGHLY SIGNIFICANT DEPRESSION IN THE FEED CONVERSIONS OF THE 20-DAY-OLD CHICKS.

KIDNEY ALKALINE PHOSPHATASE LEVELS WERE DETERMINED FOR CHICKS FED THE VARYING LEVELS OF SUPPLEMENTAL MOLYBDENUM. AS MAY BE SEEN FROM THESE DETERMINATIONS (TABLE XXX) THERE WAS AN APPARENT INCREASE IN THE LEVELS OF THIS ENZYME, ESPECIALLY AT THE HIGHER SUPPLEMENTATION LEVELS.

TABLE XXIX

ANALYSIS OF VARIANCE ON FEED CONVERSIONS OF CHICKS, FROM NON-SUPPLEMENTED DAMS, FED PURIFIED RATION WITH ADDED MOLYBDENUM

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	78	-	-
BETWEEN MOLYBDENUM LEVELS	10	298	2.10*
0 VS 1 PPM MOLYBDENUM	1	229	1.61
0 VS 2 PPM MOLYBDENUM	1	221	1.56
0 VS 3 PPM MOLYBDENUM	1	50	0.35
0 VS 5 PPM MOLYBDENUM	1	406	2.86
0 VS 10 PPM MOLYBDENUM	1	91	0.64
0 VS 50 PPM MOLYBDENUM	1	83	0.58
0 VS 100 PPM MOLYBDENUM	1	89	0.63
0 VS 500 PPM MOLYBDENUM	1	7	0.05
0 VS 1000 PPM MOLYBDENUM	1	610	4.29*
0 VS 2000 PPM MOLYBDENUM	1	1041	7.53**
ERROR	68	142	-

AT THE 3000 PPM MOLYBDENUM SUPPLEMENTATION LEVEL, BELIEVED TO BE IN THE TOXIC RANGE, THE ENZYME LEVEL WAS MORE THAN THREE TIMES GREATER THAN AT LOW SUPPLEMENTATION LEVELS.

III. PRACTICAL BROILER RATION STUDIES WITH CHICKS FROM TUNGSTATE SUPPLEMENTED DAMS.

THE METHOD OF REARING THE CHICKS IN THIS STUDY WAS THE SAME AS DISCUSSED IN PREVICUS SECTIONS. THE BASAL RATION WAS THE PRACTICAL CORN-SOYBEAN MEAL BROILER RATION. NINE LEVELS OF SUPPLEMENTAL

TABLE XXX

ALKALINE PHOSPHATASE LEVELS OF KIDNEY TISSUES OF CHICKS, FROM NON-SUPPLEMENTED DAMS, FED PURIFIED RATION PLUS MOLYBDENUM

ADDED MOLYBDENUM (PPM)	GAIN (GRAMS)	NET YIELD OF ALKALINE PHOSPHATASE*
0	177	30.2775
2	171	29.8718
3	172	31.5619
5	173	31.9992
50	182	40.9213
100	182	41.4850
500	175	44.3089
1000	142	54.2775
2000	99	67.9600
3000	68	103.2559

*VALUES EXPRESSED AS MILLIGRAMS PHOSPHORUS PER GRAM KIDNEY TISSUE (WET WEIGHT).

molybdenum ranging form 0.5 to 1000 ppm were tested. The chicks tested were from dams fed the tungstate supplemented breeder ration.

The purpose of this study was to determine the effect of various levels of supplemental molybdenun on chicks, from supplemented dams, when fed a practical broiler ration. This study involved 18 lots of 12 chicks each. The gains and feed conversions for the 20-day trial period are shown in Table XXXI. An analysis of variance was made on the gain data and is presented in Table XXXII.

TABLE XXXI

Gains and Feed Conversions of Chicks, From Tungstate Supplemented Dams, Fed A Practical Broiler Ration Plus Molybdenum

Added Molybdenum	Gain	Feed Conversion
(ppm)	(Grams)	
0	207	1.65
0.5	215	1.66
1.0	221	1.62
5.0	218	1.67
10.0	226	1.66
50.0	210	1.63
100.0	210	1.67
500.0	164	1.74
1000.0	122	2.31

TABLE XXXII

ANALYSIS OF VARIANCE ON GAINS OF CHICKS, FROM TUNGSTATE SUPPLEMENTED DAMS, FED A PRACTICAL BROILER RATION PLUS MOLYBDENUM

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	207	-	-
BETWEEN MOLYBDENUM LEVELS	8	26425	58.90**
0 VS 0.5 PPM MOLYBDENUM	1	764	1.70
0 VS 1.0 PPM MOLYBDENUM	1	2237	4.98*
0 VS 5.0 PPM MOLYBDENUM	1	1404	3.13
0 VS 10.0 PPM MOLYBDENUM	1	4341	9.67**
0 VS 50.0 PPM MOLYBDENUM	1	88	0.20
0 VS 100.0 PPM MOLYBDENUM	1	52	0.12
0 VS 500.0 PPM MOLYBDENUM	1	25419	56.61**
0 VS 1000.0 PPM MOLYBDENUM	1	99597	221.82**
ERROR	199	449	-

*SIGNIFICANT AT 0.05 LEVEL OF PROBABILITY

**SIGNIFICANT AT 0.01 LEVEL OF PROBABILITY

IT WAS FOUND THAT THE ADDITION OF 0.5 PPM MOLYBDENUM HAD NO REAL EFFECT ON THE GAINS. THIS DIFFERENCE WAS SLIGHT AND MIGHT CONCEIVABLY HAVE BEEN SIGNIFICANT IF LARGER NUMBERS HAD BEEN USED. MOLYBDENUM ADDITION LEVELS OF ONE AND FIVE PPM GAVE STATISTICALLY SIGNIFICANT INCREASES IN THE RATE OF GAIN, WHILE THE ADDITION OF 10 PPM GAVE A HIGHLY SIGNIFICANT GROWTH STIMULATION. NO EFFECT ON GAIN WAS NOTED FROM THE ADDITION OF EITHER 50 OR 100 PPM MOLYBDENUM. INCLUSION OF

TABLE XXXIII

ANALYSIS OF VARIANCE ON FEED CONVERSION DATA OF CHICKS, FROM TUNGSTATE SUPPLEMENTED DAMS, FED PRACTICAL BROILER RATION PLUS MOLYBDENUM

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	23		
BETWEEN MOLYBDENUM LEVELS	8	102.5	11.82**
0 VS 0.5 PPM MOLYBDENUM	1	3.0	0.35
0 VS 1.0 PPM MOLYBDENUM	1	18.0	2.07
0 VS 5.0 PPM MOLYBDENUM	1	5.0	0.58
0 VS 10.0 PPM MOLYBDENUM	1	2.0	0.23
0 VS 50.0 PPM MOLYBDENUM	1	7.0	0.81
0 VS 100.0 PPM MOLYBDENUM	1	2.0	0.23
0 VS 500.0 PPM MOLYBDENUM	1	102.0	11.76**
0 VS 1000.0 PPM MOLYBDENUM	1	594.0	68.51**
ERROR	15	8.7	

** SIGNIFICANT AT 0.01 LEVEL OF PROBABILITY

500 PPM AND 1000 PPM SUPPLEMENTAL MOLYBDENUM CAUSED A HIGHLY SIGNIFICANT DEPRESSION IN THE RATES OF GAIN.

A STATISTICAL ANALYSIS WAS MADE ON THE FEED CONVERSION DATA. THE ANALYSIS OV VARIANCE IS SHOWN IN TABLE XXXIII. THE ANALYSIS REVEALED THAT THE ADDITIONS OF 0.5, 1, 5, 10, AND 100 PPM MOLYBDENUM TO THE PRACTICAL-TYPE BROILER RATION HAD NO SIGNIFICANT EFFECT ON THE FEED CONVERSION OF THE 20-DAY OLD CHICKS. APPARENTLY THE TOXIC LEVEL OF

TABLE XXXIV

URIC ACID SYNTHESIS AS DETERMINED FROM VARIOUS TISSUES OF CHICKS, FROM TUNGSTATE SUPPLEMENTED DAMS, FED A PRACTICAL BROILER RATION PLUS MOLYBDENUM ADDITIONS

ADDED MOLYBDENUM	GAIN	10-MINUTE ENDOGENOUS OXIDATION	30-MINUTE ENDOGENOUS OXIDATION	NET 20-MINUTE ENZYMATIC OXIDATION
(PPM)	(GRAMS)			
		LIVER*		
0	201	0.22	0.59	5.02
50	207	0.36	0.54	5.11
100	209	0.48	0.64	5.37
500	164	0.42	0.60	5.34
1000	122	0.13	0.52	4.49
		KIDNEY*		
0	201	0.42	0.56	3.74
50	207	0.42	0.50	4.52
100	209	0.53	0.61	4.32
500	164	0.48	0.69	4.65
1000	122	0.51	0.80	4.10

* DETERMINATIONS ARE EXPRESSED AS MILLIGRAMS URIC ACID PER GRAM OF TISSUE (WET WEIGHT).

MOLYBDENUM SUPPLEMENTATION WAS REACHED WHEN 500 PPM MOLYBDENUM WAS ADDED, AS THIS LEVEL CAUSED A HIGHLY SIGNIFICANT DEPRESSION IN THE FEED CONVERSION. THE INCLUSION OF 1000 PPM CAUSED A MORE MARKED DEPRESSION OF THE FEED CONVERSION IN THESE CHICKS.

AT THE END OF THE TRIAL, SAMPLES OF BIRDS FROM GROUPS FED THE

PRACTICAL BROILER RATION WITH MOLYBDENUM SUPPLEMENTATIONS OF 0, 50, 100, 500, AND 1000 PPM WERE SLAUGHTERED AND THE RATE OF URIC ACID SYNTHESIS WAS MEASURED ON LIVER AND KIDNEY TISSUES. THESE URIC ACID SYNTHESIS RATES ARE SHOWN IN TABLE XXXIV. AS MAY BE SEEN BY THESE DATA, THERE APPEARED TO BE NO DEFINITE CHANGE EFFECTED BY THE DIFFERENT LEVELS OF SUPPLEMENTAL MOLYBDENUM. A STATISTICAL ANALYSIS FAILED TO REVEAL ANY REAL DIFFERENCES OCCURRING WITHIN THESE DATA. THIS IS IN AGREEMENT WITH THE FINDINGS WITH THE CHICKS FROM TUNGSTATE SUPPLEMENTED DAMS, FED A PURIFIED DIET PLUS VARIOUS LEVELS OF MOLYBDENUM ADDITION WHICH WAS DISCUSSED IN A PREVIOUS SECTION.

IV. Practical Broiler Ration Studies With Chicks From Non-Supplemented Dams

THE PROCEDURE USED IN THE REARING OF THE 20-DAY-OLD CHICKS WAS THE SAME AS WAS FOLLOWED PREVIOUSLY. THE PURPOSE OF THIS STUDY WAS TO DETERMINE THE EFFECT OF VARIOUS LEVELS OF SUPPLEMENTAL MOLYBDENUM ON CHICKS, FROM NON-SUPPLEMENTED DAMS, WHEN FED A PRACTICAL BROILER RATION. THIS STUDY INVOLVED 18 LOTS OF 11 CHICKS EACH. AS IN THE PREVIOUS TRIAL, THE EFFECTS OF NINE LEVELS OF SUPPLEMENTAL MOLYBDENUM WERE TESTED. THE RESULTS ARE SHOWN IN TABLE XXXV.

AN ANALYSIS OF VARIANCE OF THE GAIN DATA (TABLE XXXVI) REVEALED THAT HIGHLY SIGNIFICANT DIFFERENCES EXISTED. WHEN THE VARIOUS LEVELS OF ADDED MOLYBDENUM WERE STATISTICALLY COMPARED WITH THE BASAL RATION, IT WAS FOUND THAT NO REAL DIFFERENCES EXISTED IN THE GAIN PRODUCED BY THE BASAL RATION AND THAT FROM THE PRACTICAL BROILER RATION PLUS SUPPLEMENTAL MOLYBDENUM LEVELS OF 1, 5, 10, 50 AND 100 PPM. HIGHER ADDITIONS OF MOLYBDENUM, I.E. 500 AND 1000 PPM, CAUSED A HIGHLY SIGNIFICANT DEPRESSION IN THE RATE OF GAIN WHEN COMPARED TO THE GAIN

TABLE XXXV

GAIN AND FEED CONVERSION DATA OF CHICKS, FROM NON-SUPPLEMENTED DAMS, FED A PRACTICAL BROILER RATION WITH MOLYBDENUM SUPPLEMENTATION

ADDED MOLYBDENUM (PPM)	GAIN (GRAMS)	FEED CONVERSION
0	221	1.55
0.5	221	1.55
1.0	225	1.54
5.0	221	1.57
10.0	220	1.53
50.0	221	1.60
100.0	220	1.60
500.0	170	1.65
1000.0	115	1.88

SUPPORTED BY THE BASAL RATION. AGAIN, AS IN PREVIOUS STUDIES USING THESE LEVELS OF ADDED MOLYBDENUM, IT HAS BEEN FOUND THAT IN ALL CASES THE ADDITION OF 1000 PPM MOLYBDENUM APPEARED TO HAVE A TOXIC EFFECT AS EVIDENCED BY THE RATE OF GAIN AND IN MOST CASES A 500 PPM SUPPLEMENTATION HAS A MARKED EFFECT.

AGAIN, ATTENTION IS CALLED TO THE FACT THAT CHICKS FROM TUNGSTATE SUPPLEMENTED DAMS, WHEN FED 10 PPM MOLYBDENUM, WERE FOUND TO HAVE A HIGHER RATE OF GAIN THAN CHICKS FROM NON-SUPPLEMENTED DAMS, WHEN FED ANY LEVEL OF SUPPLEMENTAL MOLYBDENUM. THIS ADDS STRENGTH TO THE POSTULATE, REPORTED IN THE PURIFIED RATION SERIES, THAT THERE APPEARS

TABLE XXXVI

ANALYSIS OF VARIANCE ON GAIN DATA OF CHICKS, FROM NON-SUPPLEMENTED DAMS, FED A PRACTICAL BROILER RATION WITH MOLYBDENUM SUPPLEMENTATION

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	197		
BETWEEN LEVELS OF MOLYBDENUM	8	34726	52.70**
0 VS 0.5 PPM MOLYBDENUM	1	4	0.01
0 VS 1 PPM MOLYBDENUM	1	567	0.86
0 VS 5 PPM MOLYBDENUM	1	3	0.01
0 VS 10 PPM MOLYBDENUM	1	37	0.06
0 VS 50 PPM MOLYBDENUM	1	8	0.01
0 VS 100 PPM MOLYBDENUM	1	446	0.68
0 VS 500 PPM MOLYBDENUM	1	34296	52.04**
0 VS 1000 PPM MOLYBDENUM	1	155424	235.57**
ERROR	189	659	

** SIGNIFICANT AT 0.01 LEVEL OF PROBABILITY

A SYNERGISTIC EFFECT BETWEEN MOLYBDENUM AND TUNGSTEN, AS EVIDENCED BY THIS INCREASED RATE OF GAIN BOTH IN THE PRACTICAL AND PURIFIED RATION STUDIES.

A STATISTICAL ANALYSIS WAS MADE ON THE FEED CONVERSION DATA FROM THIS STUDY. IT WAS FOUND THAT NO STATISTICAL DIFFERENCES EXISTED IN THE FEED CONVERSION RATES. IT IS TRUE THAT THE HIGHER TWO LEVELS OF MOLYBDENUM SUPPLEMENTATION APPEARED TO GIVE A DEPRESSION IN THE FEED CONVERSION RATE BUT, BECAUSE OF THE RELATIVELY SMALL NUMBER OF GROUPS

FED EACH LEVEL AND THE VARIATION THAT EXISTED WITHIN THESE RESULTS, THESE HIGHER FEED CONVERSION VALUES WERE NOT STATISTICALLY SIGNIFICANT.

IN ORDER TO DETERMINE WHETHER THE TUNGSTATE SUPPLEMENTED DAMS EXHIBITED AN EFFECT ON THE GAIN OR FEED CONVERSION RATE OF THESE 20-DAY-OLD CHICKS, ANALYSES OF VARIANCE WERE MADE COMPARING BOTH THE GAIN AND FEED CONVERSION RATES BETWEEN THE CHICKS FROM TUNGSTATE SUPPLEMENTED AND NON-SUPPLEMENTED DAMS. IN BOTH CASES, HIGHLY SIGNIFICANT DIFFERENCES WERE FOUND, THUS INDICATING THAT THE TUNGSTATE FED TO THE DAMS HAD THE EFFECT OF DECREASING BOTH THE RATE OF GAIN AND THE FEED CONVERSION OF CHICKS TO AT LEAST 20-DAYS OF AGE.

AT THE COMPLETION OF THE TRIAL, BIRDS WERE SELECTED FROM GROUPS FED LEVELS OF 0, 50, 100, 500, AND 1000 PPM MOLYBDENUM. THE BIRDS WERE SLAUGHTERED AND URIC ACID SYNTHESIS (AS DESCRIBED IN APPENDIX II) DETERMINATIONS WERE MADE ON LIVER AND KIDNEY TISSUES USING XANTHINE AS THE SUBSTRATE. THESE DATA ARE PRESENTED IN TABLE XXXVII. AGAIN, AS IN THE PREVIOUS CASES OF SIMILAR URIC ACID DETERMINATIONS, THE MOLYBDENUM ADDED TO THE DIETS DID NOT EXHIBIT A DEFINITE EFFECT. AN ANALYSIS OF VARIANCE FAILED TO REVEAL ANY SIGNIFICANT DIFFERENCES IN THESE DATA. IN A COMPARISON OF THESE DATA WITH THE DATA FROM TUNGSTATE SUPPLEMENTED CHICKS IN THE PREVIOUS TRIAL, IT WAS FOUND THAT THERE WAS AN OVER-ALL INCREASE IN THE URIC ACID SYNTHESIS IN TISSUES FROM THE NON-SUPPLEMENTED CHICKS.

Summary

IT WAS FOUND THAT THERE WAS A CARRY-OVER OF TUNGSTEN FROM THE DAM (FED SUPPLEMENTAL TUNGSTEN) TO THE CHICKS AND THAT THE KIDNEY CONTAINED LARGE AMOUNTS OF TUNGSTEN COMPARED TO CHICKS FROM

TABLE XXXVII

URIC ACID SYNTHESIS AS DETERMINED FROM VARIOUS TISSUES OF CHICKS, FROM NON-SUPPLEMENTED DAMS, FED A PRACTICAL BROILER RATION WITH MOLYBDENUM SUPPLEMENTATION

ADDED MOLYBDENUM (PPM)	GAIN (GRAMS)	10-MINUTE ENDOGENOUS OXIDATION	30-MINUTE ENDOGENOUS OXIDATION	NET 20-MINUTE ENZYMATIC OXIDATION
		LIVER*		
0	222	0.11	0.22	8.33
50	221	0.18	0.29	8.14
100	227	0.06	0.13	7.70
500	170	0.11	0.03	6.43
1000	113	0.08	0.20	7.27
		KIDNEY*		
0	222	0.67	0.84	6.74
50	221	0.71	0.76	6.68
100	227	0.52	0.61	8.37
500	170	0.44	0.47	7.62
1000	113	0.52	0.64	8.38

* DETERMINATIONS EXPRESSED AS MILLIGRAMS URIC ACID PER GRAM TISSUE (WET WEIGHT).

NON-SUPPLEMENTED DAMS. WHEN CHICKS FROM THE TUNGSTATE SUPPLEMENTED HENS, WERE FED EITHER THE PURIFIED RATION OR THE PRACTICAL BROILER RATION THEY GREW AT A SIGNIFICANTLY SLOWER RATE THAN DID CHICKS FROM NON-SUPPLEMENTED DAMS. WHEN MOLYBDENUM WAS ADDED TO THE RATION IN SUPPLEMENTAL LEVELS UP TO 100 PPM THERE WAS NO EFFECT ON GROWTH OR FEED CONVERSION IN THE CASE OF CHICKS FROM NON-SUPPLEMENTED DAMS; HOWEVER, THE CHICKS FROM TUNGSTATE SUPPLEMENTED DAMS, MADE SIGNIFICANTLY GREATER GAINS OVER THOSE OBTAINED ON THE BASAL RATIONS WHEN SUPPLEMENTED WITH MOLYBDENUM UP TO 100 PPM. IT WAS OF INTEREST TO FIND THAT THESE CHICKS ACTUALLY MADE GREATER GAINS, WHEN FED THE BASAL RATIONS WITH 10 AND 50 PPM MOLYBDENUM, THAN DID CHICKS FROM NON-SUPPLEMENTED DAMS REGARDLESS OF THE LEVELS OF MOLYBDENUM SUPPLEMENTATION. THIS SUGGESTED THAT THERE MIGHT BE A SYNERGISTIC EFFECT OF MOLYBDENUM AND TUNGSTEN FOR GROWING CHICKS. THE ADDITION OF 500 PPM MOLYBDENUM TO EITHER THE PURIFIED DIET OR THE PRACTICAL BROILER RATION RESULTED IN A SIGNIFICANT DEPRESSION IN THE RATES OF GAIN AND FEED CONVERSIONS OF CHICKS FROM THE TUNGSTATE SUPPLEMENTED DAMS. THIS APPARENT "TOXIC LEVEL" WAS NOT REACHED, IN THE CASE OF THE CHICKS FROM THE NON-SUPPLEMENTED DAMS, UNTIL THE MOLYBDENUM SUPPLEMENTATION REACHED 1000 PPM IN EITHER OF THE BASAL RATIONS USED.

RESULTS OBTAINED FROM MEASUREMENTS OF URIC ACID SYNTHESIS IN THE LIVER AND KIDNEY DID NOT REFLECT ANY DIFFERENCE IN FEED SUPPLEMENTATION OR RATE OF GAIN. ALKALINE PHOSPHATASE ACTIVITIES APPEARED TO INCREASE WITH THE INCREASING LEVELS OF MOLYBDENUM SUPPLEMENTATION. WHEN THE SO CALLED "TOXIC LEVELS" WERE REACHED, AS MEASURED BY GAINS AND FEED

CONVERSIONS, THERE WERE MARKED INCREASES IN THE LEVELS OF ALKALINE PHOSPHATASE ACTIVITY. IT APPEARS THAT INCREASES IN A STRESS CONDITION MIGHT HAVE RESULTED IN THESE INCREASED ENZYME LEVELS.

D. Tungstate Supplementation of Chick Diets

In the previous study it appeared that tungsten and molybdenum might exert a synergistic effect in growing chicks. In order to test this postulate, it was deemed appropriate to undertake a study in which various levels of tungsten could be tested in the rations, with and without the presence of supplemental levels of molybdenum.

This study involved chicks from tungstate supplemented and non-supplemented White Plymouth Rock hens as well as commercial Vantress-White Plymouth Rock chicks. Arrangements were made with a commercial hatchery to supply chicks from the same flock for the time necessary to conduct this series of trials using the Vantress-Cross chicks. The method of raising the chicks was the same as discussed in previous studies, except that all chicks were placed on the basal rations for a period of one week. At the end of this period the chicks were weighed individually and allotted into groups. This procedure was used in an attempt to reduce the variation within each treatment. Twenty-day gains and feed conversions were the criteria used in this series of trials.

A three by three factorial type experiment was designed to study the various levels of molybdenum and tungsten in these chicks. All possible combinations of zero, one, and ten ppm tungsten and zero, one, and ten ppm molybdenum were tested. Samples of birds from each treatment were slaughtered and uric acid synthesis and alkaline phosphatase activities were determined at the end of the trials.

1. Purified Ration Studies With Chicks From Tungstate Fed Hens

Eighteen lots of 10 chicks each were used in this study. The rate of gain and feed conversions are presented in Table XXXVIII.

TABLE XXXVIII

GAIN AND FEED CONVERSION DATA ON CHICKS, FROM TUNGSTATE SUPPLEMENTED DAMS, FED PURIFIED RATION WITH TUNGSTEN AND MOLYBDENUM ADDITIONS

ELEMENT ADDED (PPM)		GAIN (GRAMS)	FEED CONVERSION
0 MOLYBDENUM			
	+ 0 TUNGSTEN	234	1.35
	+ 1 TUNGSTEN	242	1.37
	+ 10 TUNGSTEN	223	1.76
1 MOLYBDENUM			
	+ 0 TUNGSTEN	237	1.41
	+ 1 TUNGSTEN	253	1.43
	+ 10 TUNGSTEN	229	1.47
10 MOLYBDENUM			
	+ 0 TUNGSTEN	246	1.47
	+ 1 TUNGSTEN	254	1.35
	+ 10 TUNGSTEN	238	1.51

AN ANALYSIS OF VARIANCE WAS MADE ON THE GAINS AND IS PRESENTED IN TABLE XXXIX. THE ANALYSIS SHOWED THAT THERE WERE HIGHLY SIGNIFICANT DIFFERENCES IN THE RATE OF GAIN IN THESE TRIALS. WHEN THE EFFECTS OF THE DIFFERENT ADDITIVES WERE STATISTICALLY COMPARED, IT WAS FOUND THAT THE ADDITION OF ONE PPM OR 10 PPM TUNGSTATE HAD NO STATISTICALLY SIGNIFICANT EFFECT WHEN ADDED TO THE BASAL RATION. THE ADDITION OF ONE PPM MOLYBDENUM HAD NO EFFECT; HOWEVER THE SUPPLEMENTATION OF THE PURIFIED RATION WITH ONE PPM OF MOLYBDENUM AND ONE PPM OF TUNGSTEN CAUSED A HIGHLY SIGNIFICANT INCREASE IN THE RATE OF GAIN. THE ADDITION OF ONE PPM MOLYBDENUM IN THE PRESENCE OF 10 PPM TUNGSTATE HAD NO REAL

TABLE XXXIX

ANALYSIS OF VARIANCE ON GAIN OF CHICKS, FROM TUNGSTATE SUPPLEMENTED DAMS, FED PURIFIED RATION WITH MOLYBDENUM AND TUNGSTEN ADDITIONS

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	177	-	-
BETWEEN ADDITIVE TREATMENTS	8	1006.0	2.41*
TUNGSTATE	2	84.0	0.20
MOLYBDENUM	2	6040.0	14.48**
0 VS 1 PPM MOLYBDENUM	1	43.0	0.01
0 VS 10 PPM MOLYBDENUM	1	1816.0	4.35*
TUNGSTATE X MOLYBDENUM	4	15585.0	37.38**
0 VS 1 PPM Mo# + 1 PPM W#	1	2143.0	5.14*
0 VS 1 PPM Mo + 10 PPM W	1	168.0	0.40
0 VS 10 PPM Mo + 1 PPM W	1	2283.0	5.47*
0 VS 10 PPM Mo + 10 PPM W	1	42.0	0.01
ERROR	169	417.0	-

*SIGNIFICANT AT 0.05 LEVEL OF PROBABILITY

**SIGNIFICANT AT 0.01 LEVEL OF PROBABILITY

#Mo=MOLYBDENUM; W=TUNGSTEN

EFFECT ON GAIN WHEN COMPARED TO THE BASAL RATION. AS HAS BEEN FOUND IN PREVIOUS TRIALS, THE ADDITION OF 10 PPM MOLYBDENUM PRODUCED A SIGNIFICANT INCREASE IN THE RATE OF GAIN. WHEN ONE PPM TUNGSTATE WAS ADDED TO THE RATION THAT CONTAINED 10 PPM MOLYBDENUM, A HIGHLY SIGNIFICANT INCREASE IN THE RATE OF GAIN WAS FOUND. THE SUPPLEMENT

OF 10 PPM MOLYBDENUM AND 10 PPM TUNGSTEN IN THE PURIFIED RATION DID NOT IMPROVE THE RATE OF GAIN COMPARED TO THE BASAL RATION. THE EFFECT OF THE TUNGSTEN IN THIS RATION WAS MOST INTERESTING BECAUSE ALL PREVIOUS REPORTS STATED THAT TUNGSTEN WAS AN ANTAGONIST TO MOLYBDENUM IN NUTRITIONAL STUDIES.

AN ANALYSIS OF VARIANCE WAS MADE ON THE FEED CONVERSION DATA (TABLE XL). THE ANALYSIS REVEALED THAT HIGHLY SIGNIFICANT DIFFERENCES EXISTED WITHIN THIS TRIAL. IT WAS FOUND THAT THE ADDITION OF 10 PPM TUNGSTATE RESULTED IN A HIGHLY SIGNIFICANT DEPRESSION IN THE FEED CONVERSIONS. ALTHOUGH SOME OF THE OTHER ADDITIVES AFFECTED THE FEED CONVERSION, THESE DIFFERENCES WERE NOT STATISTICALLY SIGNIFICANT. THESE MIGHT HAVE BEEN SIGNIFICANT IF MORE OBSERVATIONS HAD BEEN RECORDED. AT THE END OF THE TRIAL PERIOD TWO BIRDS FROM EACH TREATMENT WERE SLAUGHTERED. URIC ACID SYNTHESIS WAS MEASURED ON LIVER AND KIDNEY TISSUES. THE RESULTS OF THESE DETERMINATIONS ARE SHOWN IN TABLE XLI. THERE APPEARED TO BE NO EFFECT ON THE RATE OF URIC ACID SYNTHESIS FOR ANY OF THE SUPPLEMENTAL LEVELS OF MOLYBDENUM OR TUNGSTEN, ALTHOUGH THERE WERE SIGNIFICANT DIFFERENCES IN THE RATES OF GAIN.

II. Purified Ration Studies With Chicks From Non-Supplemented Dams

THE SAME FACTORIAL EXPERIMENTAL DESIGN WAS USED AS WAS DESCRIBED IN SECTION D-I. THE PURPOSE OF THIS TRIAL WAS TO DETERMINE THE EFFECT OF VARIOUS LEVELS OF MOLYBDENUM AND TUNGSTEN, BOTH TOGETHER AND SEPARATELY, IN THE PURIFIED DIET FED TO CHICKS FROM NON-SUPPLEMENTED WHITE PLYMOUTH ROCK HENS. THE CRITERIA EMPLOYED WERE 20-DAY GAIN, FEED CONVERSION AND URIC ACID SYNTHESIS. THE GAIN AND FEED CONVERSION DATA ARE PRESENTED IN TABLE XLII.

TABLE XL

ANALYSIS OF VARIANCE ON FEED CONVERSIONS OF CHICKS, FROM TUNGSTEN FED DAMS, FED PURIFIED RATION PLUS SUPPLEMENTAL MOLYBDENUM AND TUNGSTEN

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	17	-	-
BETWEEN ADDITIVE TREATMENTS	8	711.0	26.80**
TUNGSTATE	2	487.3	18.04**
0 VS 1 PPM TUNGSTATE	1	5.0	0.20
0 VS 10 PPM TUNGSTATE	1	328.0	12.14**
MOLYBDENUM	2	27.8	1.03
TUNGSTATE X MOLYBDENUM	4	157.6	5.84*
0 VS 1 PPM Mo# + 1 PPM W#	1	11.0	0.40
0 VS 1 PPM Mo + 10 PPM W	1	26.0	0.99
0 VS 10 PPM Mo + 1 PPM W	1	1.0	0.03
0 VS 10 PPM Mo + 10 PPM W	1	50.0	1.85
ERROR	10	27.0	-

**SIGNIFICANT AT 0.01 LEVEL OF PROBABILITY

#Mo=MOLYBDENUM; W=TUNGSTEN.

A STATISTICAL ANALYSIS OF VARIANCE ON THE RATE OF GAIN IS PRESENTED IN TABLE XLIII. THE ANALYSIS REVEALED THAT HIGHLY SIGNIFICANT DIFFERENCES EXISTED WITHIN THE TRIAL. IT WAS FOUND BY STATISTICAL COMPARISONS THAT THE ADDITION OF ONE PPM TUNGSTEN HAD NO REAL EFFECT ON THE 20-DAY GROWTH RATE, BUT THAT THE ADDITION OF 10 PPM TUNGSTEN TO THE RATION CAUSED A HIGHLY SIGNIFICANT DEPRESSION OF GROWTH RATE. THE

TABLE XLI

URIC ACID DETERMINATIONS ON TISSUES ON CHICKS, FROM TUNGSTATE DAMS, FED A PURIFIED DIET PLUS MOLYBDENUM AND TUNGSTEN SUPPLEMENTS

ELEMENT ADDED (PPM)	10-MINUTE ENDOGENOUS OXIDATION	30-MINUTE ENDOGENOUS OXIDATION	NET 20-MINUTE ENZYMATIC OXIDATION[1]
	LIVER		
0 Mo*+ 0 W#	0.26	0.27	5.28
0 Mo + 1 W	0.28	0.30	6.84
0 Mo + 10 W	0.22	0.25	5.68
1 Mo + 0 W	0.13	0.17	6.12
1 Mo + 1 W	0.14	0.15	5.81
1 Mo + 10 W	0.11	0.17	4.81
10 Mo + 0 W	0.27	0.28	6.67
10 Mo + 1 W	0.27	0.27	8.18
10 Mo + 10 W	0.24	0.25	7.69
	KIDNEY		
0 Mo + 0 W	0.50	0.55	6.50
0 Mo + 1 W	0.64	0.74	6.00
0 Mo + 10 W	0.51	0.53	6.65
1 Mo + 0 W	0.39	0.44	5.68
1 Mo + 1 W	0.30	0.38	6.09
1 Mo + 10 W	0.29	0.36	4.78
10 Mo + 0 W	0.56	0.69	7.63
10 Mc + 1 W	0.51	0.59	7.10
10 Mo + 10 W	0.50	0.52	6.08

[1] URIC ACID DETERMINATIONS ARE EXPRESSED AS MILLIGRAMS URIC ACID PER GRAM TISSUE (WET WEIGHT)

* Mo = MOLYBDENUM # W = TUNGSTEN

TABLE XLII

GAIN AND FEED CONVERSION DATA OF CHICKS, FROM NON-SUPPLEMENTED DAMS, FED A PURIFIED RATION WITH TUNGSTEN AND MOLYBDENUM ADDITIONS

ELEMENT ADDED		GAIN	FEED CONVERSION
(PPM)		(GRAMS)	
0 MOLYBDENUM	+ 0 TUNGSTEN	247	1.33
	+ 1 TUNGSTEN	250	1.38
	+ 10 TUNGSTEN	176	1.84
1 MOLYBDENUM	+ 0 TUNGSTEN	242	1.39
	+ 1 TUNGSTEN	257	1.39
	+ 10 TUNGSTEN	228	1.43
10 MOLYBDENUM	+ 0 TUNGSTEN	242	1.37
	+ 1 TUNGSTEN	260	1.31
	+ 10 TUNGSTEN	235	1.48

ADDITION OF ONE PPM MOLYBDENUM PLUS 10 PPM TUNGSTEN RESULTED IN A HIGHLY SIGNIFICANT GROWTH DEPRESSION BUT THE DEPRESSION WAS NOT AS DRASTIC AS THAT FROM 10 PPM TUNGSTATE ALONE. THE ADDITION OF 10 PPM MOLYBDENUM PLUS 10 PPM TUNGSTEN STILL RESULTED IN A SIGNIFICANT DEPRESSION IN GAIN, BUT NOT AS SEVERE AS WHEN 10 PPM TUNGSTATE WAS ADDED ALONE, OR IN THE PRESENCE OF ONE PPM MOLYBDENUM.

THE SAME TREND WAS FOUND IN THIS TRIAL AS IN THE CASE OF THE CHICKS FROM THE TUNGSTATE SUPPLEMENTED DAMS. WHEN ONE PPM MOLYBDENUM WAS ADDED IN THE PRESENCE OF ONE PPM TUNGSTEN, A SIGNIFICANT STIMULATION IN GAIN RESULTED. THIS WAS MAGNIFIED WHEN THE RATIO OF

TABLE XLIII

ANALYSIS OF VARIANCE ON GAIN OF CHICKS, FROM NON-SUPPLEMENTED DAMS, FED A PURIFIED DIET PLUS MOLYBDENUM AND TUNGSTEN SUPPLEMENTATIONS

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	143		
BETWEEN ADDITIVE TREATMENTS	8	1514	5.03**
MOLYBDENUM	2	437	1.45
TUNGSTATE	2	5245	17.40**
0 VS 1 PPM TUNGSTATE	1	175	0.58
0 VS 10 PPM TUNGSTATE	1	6160	20.49**
TUNGSTATE X MOLYBDENUM	4	7006	23.27**
0 VS 1 PPM Mo + 1 PPM W	1	1818	6.04*
0 VS 1 PPM Mo + 10 PPM W	1	4513	14.99**
0 VS 10 PPM Mo + 1 PPM W	1	2048	6.88**
0 VS 10 PPM Mo + 10 PPM W	1	1741	5.80*
ERROR	135	301	

* SIGNIFICANT AT 0.05 LEVEL OF PROBABILITY

** SIGNIFICANT AT 0.01 LEVEL OF PROBABILITY

Mo = MOLYBDENUM W = TUNGSTEN

MOLYBDENUM:TUNGSTEN WAS 10:1, AS A HIGHLY SIGNIFICANT GROWTH STIMULATION WAS OBSERVED. THE ADDITION OF ONE PPM OR 10 PPM MOLYBDENUM ALONE HAD NO REAL EFFECT. THIS WAS FOUND TO BE IN AGREEMENT WITH EARLIER RESULTS REPORTED IN THIS OVER-ALL STUDY. AGAIN THIS POINTS UP THE FACT THAT THERE APPEARS TO BE A SYNERGISTIC EFFECT OF TUNGSTEN AND MOLYBDENUM IN

TABLE XLIV

ANALYSIS OF VARIANCE ON FEED CONVERSION OF CHICKS, FROM NON-SUPPLEMENTED DAMS, FED A PURIFIED DIET WITH MOLYBDENUM AND TUNGSTEN SUPPLEMENTATION

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	17		
BETWEEN ADDITIVES	8	284.0	2.39
TUNGSTATE	2	70.4	0.59
MOLYBDENUM	2	104.0	0.88
TUNGSTATE X MOLYBDENUM	4	207.4	1.72
ERROR	9	119.0	

DIETS OF GROWING CHICKS. THIS SYNERGISTIC EFFECT WAS FOUND IN CHICKS REGARDLESS OF THE TREATMENT OF DAMS FROM WHICH THEY CAME.

AN ANALYSIS OF VARIANCE WAS MADE ON THE FEED CONVERSIONS AND IS PRESENTED IN TABLE XLIV. THE ANALYSIS REVEALED NO SIGNIFICANT DIFFERENCES EXISTED IN THE FEED CONVERSIONS. HAD MORE GROUPS BEEN USED IN THIS STUDY, THERE IS LITTLE DOUBT THAT THE FEED CONVERSIONS OF THE GROUPS FED 10 PPM TUNGSTEN WOULD HAVE BEEN STATISTICALLY SIGNIFICANT.

TWO BIRDS FROM EACH GROUP WERE SELECTED AND URIC ACID SYNTHESIS WAS MEASURED IN LIVER AND KIDNEY TISSUES. THE RESULTS OF THESE DETERMINATIONS ARE SHOWN IN TABLE XLV. AGAIN, THERE APPEARED TO BE NO CORRELATION BETWEEN GROWTH AND THESE DETERMINATIONS. IT WAS DECIDED TO DISCONTINUE THE URIC ACID DETERMINATIONS, SINCE NO CORRELATION WITH THE RATE OF GAIN, FEED CONVERSIONS, MOLYBDENUM OR TUNGSTEN ADDITIONS COULD BE FOUND.

TABLE XLV

URIC ACID DETERMINATIONS OF TISSUES FROM CHICKS, FROM NON-SUPPLEMENTED DAMS, FED A PURIFIED DIET WITH MOLYBDENUM AND TUNGSTEN ADDITIONS

ELEMENT ADDED (PPM)	10-MINUTE ENDOGENOUS OXIDATION	30-MINUTE ENDOGENOUS OXIDATION	NET 20-MINUTE ENZYMATIC OXIDATION[1]
	LIVER		
0 Mo + 0 W*	0.40	0.44	8.32
0 Mo + 1 W	0.28	0.32	8.95
0 Mo + 10 W	0.18	0.21	8.32
1 Mo + 0 W	0.25	0.29	7.58
1 Mo + 1 W	0.19	0.30	7.74
1 Mo + 10 W	0.14	0.24	8.42
10 Mo + 0 W	0.26	0.50	8.94
10 Mo + 1 W	0.30	0.54	9.38
10 Mo + 10 W	0.22	0.41	7.31
	KIDNEY		
0 Mo + 0 W	0.60	0.62	7.41
0 Mo + 1 W	0.40	0.46	8.21
0 Mo + 10 W	0.42	0.51	9.26
1 Mo + 0 W	0.51	0.62	7.37
1 Mo + 1 W	0.69	0.79	9.47
1 Mo + 10 W	0.40	0.64	8.86
10 Mo + 0 W	0.88	1.49	10.14
10 Mo + 1 W	0.77	1.42	7.85
10 Mo + 10 W	0.77	1.29	9.60

[1] URIC ACID DETERMINATION EXPRESSED AS MILLIGRAMS URIC ACID PER GRAM TISSUE (WET WEIGHT).

* Mo = MOLYBDENUM W = TUNGSTEN

III. Practical Broiler Ration Studies With Commercial Chicks

A commercial broiler strain was used in this study. The chicks were fed the practical type broiler ration presented in Table I. The gain and feed conversion data obtained in this study are presented in Table XLVI.

A statistical analysis was made on the gain data. This analysis (Table XLVII) revealed significant differences occurring within the rates of gain. By means of statistical comparisons it was found that single additions of tungsten at one or 10 ppm had no significant effect on the 20-day gains when compared to the rate of gain supported by the basal broiler ration alone. The addition of one ppm molybdenum, one ppm molybdenum plus one ppm tungsten, and 10 ppm molybdenum plus one ppm tungsten all caused a significant increase in the rate of gain. The supplementation of 10 ppm molbydenum alone or with the addition of 10 ppm tungsten had no effect on the rate of gain with chicks used in this study.

An analysis of variance was made on the feed conversion data obtained from this study. This analysis is presented in Table XLVIII. It was found that none of the additives caused an improvement of feed conversion over that obtained from the practical broiler diet with no additives. The addition of 10 ppm tungsten to the broiler ration resulted in a highly significant depression in the feed conversion rate. The addition of one ppm molybdenum plus 10 ppm tungsten gave a significant depression; it appeared as if the addition of molybdenum to this level of tungsten partially overcame this depression. This was further substantiated by the fact that the addition of 10 ppm of both molybdenum and tungsten to the practical ration resulted in no

TABLE XLVI

GAIN, FEED CONVERSION, AND ALKALINE PHOSPHATASE LEVELS OF COMMERCIAL BROILER CHICKS FED PRACTICAL-TYPE BROILER RATION WITH MOLYBDENUM AND TUNGSTEN SUPPLEMENTS

ELEMENT ADDED (PPM)	GAIN (GRAMS)	FEED CONVERSION	ALKALINE PHOSPHATASE VALUES*
0 MOLYBDENUM 0 TUNGSTEN	297	1.75	105.5023
0 MOLYBDENUM 1 TUNGSTEN	289	1.78	119.6235
0 MOLYBDENUM 10 TUNGSTEN	287	1.92	137.0818
1 MOLYBDENUM 0 TUNGSTEN	310	1.72	123.4606
1 MOLYBDENUM 1 TUNGSTEN	310	1.76	120.2787
1 MOLYBDENUM 10 TUNGSTEN	302	1.81	124.6118
10 MOLYBDENUM 0 TUNGSTEN	305	1.74	101.4896
10 MOLYBDENUM 1 TUNGSTEN	310	1.74	115.7861
10 MOLYBDENUM 10 TUNGSTEN	299	1.75	126.6695

*ALKALINE PHOSPHATASE VALUES ARE EXPRESSED IN MILLIGRAMS PHOSPHORUS PER GRAM TISSUE (WET WEIGHT).

TABLE XLVII

ANALYSIS OF VARIANCE ON GAIN OF VANTRESS-CROSS CHICKS FED A PRACTICAL BROILER RATION PLUS MOLYBDENUM AND TUNGSTEN SUPPLEMENTATIONS

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	357	-	-
BETWEEN ADDITIVES	8	1510	3.35**
TUNGSTATE	2	815	1.81
MOLYBDENUM	2	2376	5.28*
0 VS 1 PPM Mo#	1	2588	5.75*
0 VS 10 PPM Mo	1	540	1.20
TUNGSTATE X MOLYBDENUM	4	3736	8.30**
0 VS 1 PPM Mo + 1 PPM W#	1	2501	5.55*
0 VS 1 PPM Mo + 10 PPM W	1	1065	2.35
0 VS 10 PPM Mo + 1 PPM W	1	2538	5.64*
0 VS 10 PPM Mo + 10 PPM W	1	280	0.62
ERROR	349	450	-

* SIGNIFICANT AT 0.05 LEVEL OF PROBABILITY
** SIGNIFICANT AT 0.01 LEVEL OF PROBABILITY
# Mo = MOLYBDENUM; W = TUNGSTEN

DEPRESSION OF THE FEED CONVERSION DATA.

THE KIDNEY ALKALINE PHOSPHATASE VALUES ARE SHOWN IN TABLE XLVI. IT IS OF INTEREST, HOWEVER, TO NOTE THAT THERE WAS AN INCREASE IN THE ALKALINE PHOSPHATASE LEVEL EACH TIME THE LEVEL OF SUPPLEMENTAL TUNGSTEN WAS INCREASED, AND THAT THE HIGHEST ENZYME VALUE WAS OBSERVED AT THE LEVEL WITH NO ADDED MOLYBDENUM IN THE RATION.

TABLE XLVIII

ANALYSIS OF VARIANCE ON FEED CONVERSION DATA OBTAINED FROM VANTRESS-WPR CHICKS FED PRACTICAL BROILER RATION WITH TUNGSTEN AND MOLYBDENUM SUPPLEMENTATIONS

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	35	-	-
BETWEEN ADDITIVES	8	142.5	8.28**
TUNGSTATE	2	401.7	23.63**
0 VS 1 PPM W#	1	18.0	0.04
0 VS 10 PPM W	1	578.0	33.60**
MOLYBDENUM	2	22.8	1.32
TUNGSTATE X MOLYBDENUM	4	46.9	2.72
ERROR	27	17.2	-

**SIGNIFICANT AT 0.01 LEVEL OF PROBABILITY

W=TUNGSTEN

SUMMARY

RESULTS FROM THIS STUDY SHOWED THAT THE ADDITION OF ONLY TUNGSTEN TO BROILER RATIONS, EITHER PURIFIED OR PRACTICAL, HAD NO BENEFICIAL EFFECT. WHEN THE RATIO OF MOLYBDENUM TO TUNGSTEN WAS 10:1 (WT/WT) IN BOTH RATIONS, THERE WAS A SIGNIFICANT INCREASE IN GROWTH COMPARED TO THE BASAL RATIONS. THIS GIVES SUPPORT TO THE POSTULATE THAT TUNGSTATE AND MOLYBDENUM, IN PROPER RATIOS, ACT IN SOME SYNERGISTIC MANNER TO STIMULATE GROWTH. THERE WAS AN INCREASE IN THE RATE OF

GROWTH WHEN THE MOLYBDENUM:TUNGSTEN RATIO WAS 1:1 WHEN FED TO THE COMMERCIAL BROILER CHICKS FED THE PRACTICAL BROILER RATION, AND TO THE CHICKS, FROM NON-TUNGSTATE SUPPLEMENTED DAMS, WHEN FED THE PURIFIED RATION. THE ADDITION OF 10 PPM TUNGSTEN DEPRESSED THE RATE OF GROWTH IN CHICKS, REGARDLESS OF THE DAM TREATMENT, WHEN FED THE PURIFIED RATION. IT WAS INTERESTING TO NOTE THAT THE ADDITION OF 1 PPM MOLYBDENUM CAUSED AN INCREASE IN THE RATE OF GAIN WHEN FED TO THE COMMERCIAL BROILER CHICKS ON THE PRACTICAL BROILER RATION. THIS WAS THE FIRST TIME IN THIS ENTIRE STUDY THAT THE ADDITION OF MOLYBDENUM, AT ANY LEVEL, CAUSED AN INCREASE IN GAIN, EXCEPT WHEN FED TO CHICKS FROM TUNGSTATE SUPPLEMENTED DAMS.

URIC ACID SYNTHESIS, AS DETERMINED, APPEARED TO HAVE NO CORRELATION WITH THE LEVELS OF MINERALS IN THE RATIONS, THE RATES OF GAIN, OR THE FEED CONVERSION DATA. IT WAS THOUGHT THAT THE HIGHER LEVELS OF TUNGSTEN SUPPLEMENTATION OF THE RATION WOULD CAUSE A DECREASE IN THE SYNTHESIS AS IT HAS BEEN REPORTED THAT TUNGSTEN, BEING A MOLYBDATE ANTAGONIST, HAS AN INHIBITING EFFECT ON THE FORMATION OF URIC ACID.

ALKALINE PHOSPHATASE ACTIVITY LEVELS INCREASED WITH INCREASING LEVELS OF TUNGSTEN, WITH THE ADDITION OF 10 PPM CAUSING AN INCREASED ACTIVITY OF 30 PER CENT COMPARED WITH THAT OBTAINED FROM THE PRACTICAL BROILER BASAL RATION. ALKALINE PHOSPHATASE ACTIVITIES WERE NOT DETERMINED WHEN CHICKS WERE FED THE PURIFIED RATION WITH ADDED TUNGSTEN AND MOLYBDENUM.

E. Copper Supplementation of Chick Diets

It was found in the previous study that the addition of one part per million (ppm) of tungstate (as sodium tungstate) with the addition of 10 ppm of molybdenum (as sodium molybdate) gave the best results in the case of gain and feed conversion data with both rations that were used irrespective of the dam treatment on the chicks. It was decided to use these supplemental levels of tungstate and molybdenum in the basal ration to test the effect of various levels of copper in this diet. A simple titration type experimental plan was used in which levels of 0, 10, 20, 30, and 40 ppm of copper were added to the basal rations. Additional levels of 60 and 80 ppm copper were tested in the case of the practical broiler ration. As reported earlier the copper level of the practical broiler ration was some 15 ppm lower than that found in the purified ration. The rate of gain and feed conversion were used as the major criteria, although alkaline phosphatase levels were determined in an effort to see if any differences in performance of the chicks might alter the levels of this enzyme, which was determined in kidney tissue homogenates.

At the end of the 20-day trials, birds from each group were killed and alkaline phosphatase determinations were made on the tissue homogenates in the manner described in Appendix III.

The method of growing the chicks was the same as described in previous sections. Chicks from tungstate supplemented dams and non-supplemented dams were used in this study as well as Vantress-White Plymouth Rock chicks purchased from a commercial hatchery. Copper was added as cupric acetate [$Cu(C_2H_3O_2)_2.H_2O$].

1. Purified Ration Studies Involving Chicks From Tungstate Fed Dams

This study consisted of 20 lots of 10 chicks each. Each treatment was tested in four groups. The rate of gain, feed conversion data and alkaline phosphatase levels found in kidney tissue homogenates from this study are shown in Table XLIX. An analysis of variance was made on the 20-day gain data of these chicks. This analysis of variance is shown in Table L. It was found that highly significant differences occurred in the rates of gain. The components were broken out and it was found that the addition of 10 ppm copper gave no

TABLE XLIX

Rate of Gain, Feed Conversion Data and Alkaline Phosphatase Levels of Chicks, From Tungstate Fed Dams, on a Purified Diet With Copper Supplementation

Added Copper (ppm)	Gain (Grams)	Feed Conversion	Alkaline Phosphatase Levels*
0	242	1.52	44.1892
10	253	1.48	47.8655
20	258	1.44	55.0964
30	264	1.43	59.0261
40	235	1.45	79.7125

*Alkaline phosphatase expressed as milligrams phosphorus per gram kidney tissue (wet weight).

TABLE L

ANALYSIS OF VARIANCE ON RATE OF GAIN OF CHICKS, FROM TUNGSTATE SUPPLEMENTED DAMS, FED PURIFIED DIET PLUS SUPPLEMENTAL LEVELS OF COPPER

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	199	-	-
BETWEEN COPPER LEVELS	4	3403	7.58**
BASAL VS 10 PPM COPPER	1	1403	3.12
BASAL VS 20 PPM COPPER	1	2507	5.58*
BASAL VS 30 PPM COPPER	1	5244	11.67**
BASAL VS 40 PPM COPPER	1	931	2.07
ERROR	194	449	-

*SIGNIFICANT AT 0.05 LEVEL OF PROBABILITY

**SIGNIFICANT AT 0.01 LEVEL OF PROBABILITY

SIGNIFICANT INCREASE IN THE RATE OF GROWTH. HIGHER COPPER ADDITIONS OF 20 AND 30 PPM CAUSED HIGHLY SIGNIFICANT GROWTH STIMULATION, WITH THE 30 PPM LEVEL APPEARING TO GIVE MORE STIMULATION THAN THE 20 PPM SUPPLEMENTAL LEVEL OF COPPER. THE SUPPLEMENTAL ADDITION OF 40 PPM DEPRESSED GROWTH, ALTHOUGH THIS DEPRESSION WAS NOT FOUND TO BE STATISTICALLY SIGNIFICANT WHEN COMPARED TO THE BASAL RATION.

AN ANALYSIS OF VARIANCE MADE ON THE FEED CONVERSION DATA IS PRESENTED IN TABLE LI. SIGNIFICANT DIFFERENCES WERE FOUND BETWEEN THE SUPPLEMENTAL LEVELS OF COPPER. IT WAS FOUND THAT THE ADDITION OF 10 PPM SUPPLEMENTAL COPPER HAD NO EFFECT; HOWEVER, SUPPLEMENTATIONS OF 20 AND 30 PPM COPPER RESULTED IN A SIGNIFICANT IMPROVEMENT IN THE FEED

TABLE LI

ANALYSIS OF VARIANCE ON FEED CONVERSIONS OF CHICKS, FROM TUNGSTATE SUPPLEMENTED DAMS, FED PURIFIED DIET PLUS SUPPLEMENTAL LEVELS OF COPPER

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	19	-	-
BETWEEN TREATMENTS	4	53.75	5.80**
BASAL VS 10 PPM COPPER	1	41.90	4.42
BASAL VS 20 PPM COPPER	1	145.00	15.53**
BASAL VS 30 PPM COPPER	1	162.00	17.47**
BASAL VS 40 PPM COPPER	1	91.00	9.82**
ERROR	15	9.27	-

** SIGNIFICANT AT 0.01 LEVEL OF PROBABILITY

CONVERSION. THE BASAL RATION WAS THEN COMPARED WITH THE RATION CONTAINING 40 PPM SUPPLEMENTAL COPPER AND THERE WAS FOUND TO BE A REAL DIFFERENCE EXISTING BETWEEN THESE TWO RATIONS.

THE ALKALINE PHOSPHATASE ACTIVITIES APPEARED TO RISE WITH INCREASING LEVELS OF COPPER. THE INCREASED ACTIVITY WAS VERY SLIGHT WITH THE ADDITION OF 10 PPM COPPER. THE ADDITION OF 20 PPM COPPER RESULTED IN A 25 PER CENT INCREASE IN ACTIVITY WHILE THE ADDITION OF 30 PPM CAUSED AN APPARENT 33 PER CENT INCREASE IN ENZYME ACTIVITY. IT SHOULD BE NOTED THAT THIS ADDITION (30 PPM COPPER) RESULTED IN THE BEST GROWTH AND FEED CONVERSION OF THE STUDY. WHEN THE PURIFIED RATION WAS SUPPLEMENTED WITH 40 PPM COPPER, WHICH WAS SLIGHTLY DEPRESSED THE RATE OF GAIN WHEN COMPARED TO THE BASAL RATION, THERE WAS AN INCREASE IN THE ENZYME ACTIVITY OF ALMOST 80 PER CENT.

II. Purified Diet Studies With Chicks From Non-Supplemented Dams

The same procedure was used in this part of the study as was described in Section I, the only difference being the dam treatment. This portion of the study was made with chicks from non-supplemented White Plymouth Rock hens.

The rate of gain, feed conversions, and alkaline phosphatase activities of kidney tissue homogenates are presented in Table LII. An analysis of variance was made on the rate of gain of these 20-day trials (Table LIII).

The analysis of variance showed significant differences between the rates of gain for these chicks, from non-supplemented dams,

TABLE LII

Rate of Gain, Feed Conversions, and Alkaline Phosphatase Activities of Chicks, From Non-Supplemented Dams, Fed Purified Diet Plus Copper Supplementations

Added Copper (ppm)	Gain (Grams)	Feed Conversion	Alkaline Phosphatase Levels*
0	245	1.39	50.4922
10	258	1.35	50.5389
20	264	1.39	46.8984
30	268	1.38	54.6209
40	247	1.38	62.6293

*Alkaline phosphatase activity expressed as milligrams phosphorus per gram kidney tissue (wet weight).

TABLE LIII

ANALYSIS OF VARIANCE ON GAIN OF CHICKS, FROM NON-SUPPLEMENTED DAMS, FED A PURIFIED DIET WITH SUPPLEMENTAL LEVELS OF COPPER

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	197	-	-
BETWEEN COPPER TREATMENTS	4	1616	2.41*
BASAL VS 10 PPM COPPER	1	1918	2.85
BASAL VS 20 PPM COPPER	1	3783	5.63*
BASAL VS 30 PPM COPPER	1	5313	7.91**
BASAL VS 40 PPM COPPER	1	40	0.06
ERROR	193	672	-

* SIGNIFICANT AT 0.05 LEVEL OF PROBABILITY

** SIGNIFICANT AT 0.01 LEVEL OF PROBABILITY

FED THE PURIFIED RATION PLUS VARYING LEVELS OF COPPER. BY STATISTICAL COMPARISONS IT WAS FOUND THE SAME EFFECT WAS OBSERVED AS IN THE CASE OF THE CHICKS FROM THE TUNGSTATE SUPPLEMENTED DAMS; THAT IS, THE ADDITION OF 10 PPM COPPER RESULTED IN AN APPARENT INCREASE IN THE RATE OF GROWTH, WHILE SUPPLEMENTAL COPPER ADDITIONS OF 20 PPM GAVE A SIGNIFICANT INCREASE AND 30 PPM RESULTED IN A HIGHLY SIGNIFICANT INCREASE IN THE RATE OF GAIN. THE ADDITION OF 40 PPM COPPER TO THE PURIFIED CHICK DIET RESULTED IN NO GREATER GAINS THAN WERE OBTAINED ON THE UNSUPPLEMENTED BASAL RATION.

A STATISTICAL ANALYSIS OF VARIANCE ON THE FEED CONVERSIONS OBTAINED IN THIS PORTION OF THE STUDY IS PRESENTED IN TABLE LIV. THIS

TABLE LIV

ANALYSIS OF VARIANCE ON FEED CONVERSIONS OF CHICKS, FROM NON-SUPPLEMENTED DAMS, FED A PURIFIED DIET WITH VARIOUS LEVELS OF COPPER SUPPLEMENTATIONS

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	19	-	-
BETWEEN COPPER TREATMENTS	4	95.0	1.77
ERROR	15	53.6	-

ANALYSIS REVEALED THAT NO REAL DIFFERENCES EXISTED AMONG THE VARIOUS FEED CONVERSIONS. IT WAS OF INTEREST TO COMPARE THE RESPONSES OF THE CHICKS FROM THE TWO DAM TREATMENTS. STATISTICAL ANALYSES OF THE GAIN AND FEED CONVERSION DATA OBTAINED IN SECTION I AND II REVEALED NO REAL DIFFERENCES BETWEEN THE PERFORMANCE OF THE TWO GROUPS. THIS WAS OF CONSIDERABLE INTEREST, BECAUSE IN THE STUDIES INVOLVING MOLYBDENUM, THE TUNGSTATE SUPPLEMENTED RATION CONSUMED BY THE DAMS HAD SEEMINGLY EXHIBITED A DEPRESSING EFFECT IN THE CHICKS WITH REGARDS TO BOTH GROWTH AND FEED CONVERSION. APPARENTLY THE ADDITION OF COPPER TO THE RATION HAD SUCCEEDED IN OVERCOMING THIS TUNGSTATE DEPRESSING EFFECT. ONE POSSIBLE EXPLANATION OF THIS OBSERVATION IS THAT THE TUNGSTATE SUPPLEMENTATION WAS PRECIPITATING A COPPER DEFICIENCY WHICH WAS ALLEVIATED BY SUPPLEMENTAL ADDITIONS OF COPPER. IF THIS EXPLANATION WERE TRUE, ONE WOULD LOGICALLY CONCLUDE THAT TUNGSTEN AND COPPER ARE INTERRELATED IN SOME MANNER IN THEIR METABOLIC FUNCTIONS.

THE ALKALINE PHOSPHATASE ACTIVITIES WERE NOT IN COMPLETE AGREEMENT WITH THE VALUES OBTAINED WITH THE CHICKS FROM THE TUNGSTATE

SUPPLEMENTED DAMS. IN THIS PORTION OF THE STUDY THE ADDITION OF COPPER TO THE RATION APPEARED TO HAVE LITTLE EFFECT ON THE ENZYME LEVELS. AT SUPPLEMENTAL LEVELS OF 10, 20, AND 30 PPM COPPER THE ALKALINE PHOSPHATASE VALUES REMAINED RELATIVELY CONSTANT AND THE ADDITION OF 40 PPM COPPER CAUSED AN INCREASE OF ONLY ABOUT 12 PER CENT. IN CONTRAST, THE ADDITION OF 40 PPM COPPER RESULTED IN AN INCREASE OF ALMOST 80 PER CENT ENZYMATIC ACTIVITY IN THE CASE OF THE CHICKS FROM THE TUNGSTATE SUPPLEMENTED DAMS.

AN INTERESTING POSTULATE IS PROPOSED THAT ALKALINE PHOSPHATASE IS ASSOCIATED WITH KIDNEY METABOLISM OF TUNGSTEN AND COPPER. THIS MAY BE DEBATABLE HOWEVER, SINCE SUFFICIENT EVIDENCE WAS NOT OBTAINED TO SUBSTANTIATE IT. THE DRAMATIC INCREASE IN ALKALINE PHOSPHATASE VALUES ENCOUNTERED WITH THE HIGHEST LEVELS OF COPPER ADDITIONS MIGHT BE EXPLAINED ON THE BASIS OF A STRESS CONDITION BROUGHT ABOUT BY THE TOXICITY OF THE ELEMENT. THIS WOULD APPEAR TO STRENGTHEN THE POSITION THAT TUNGSTEN AND COPPER ARE RELATED AND THAT ALKALINE PHOSPHATASE IS RELATED DIRECTLY OR INDIRECTLY TO THE METABOLIC ACTIVITIES WHICH INVOLVE COPPER AND TUNGSTEN. THIS APPEARS TO BE LOGICAL SINCE THE HIGHEST ALKALINE PHOSPHATASE VALUES WERE OBTAINED WITH CHICKS HAVING THE KIDNEYS WITH THE HIGHEST TUNGSTEN CONTENT.

III. Practical Broiler Ration Studies With Vantress-White Plymouth Rock Chicks

VANTRESS-WHITE PLYMOUTH ROCK CHICKS, PURCAHSED FROM A COMMERCIAL HATCHERY, WERE USED IN THIS PORTION OF THE STUDY. THESE CHICKS WERE FED THE PRACTICAL BROILER RATION PLUS COPPER SUPPLEMENTS AT LEVELS OF 10, 20, 30, 40, 60, AND 80 PPM. THE METHODS OF BROODING AND MAINTAINING THE CHICKS WAS THE SAME AS DESCRIBED IN PREVIOUS TRIALS. FOURTEEN

TABLE LV

GAIN, FEED CONVERSIONS, AND ALKALINE PHOSPHATASE ACTIVITIES OF CHICKS, FED A PRACTICAL BROILER RATION WITH COPPER ADDITIONS

ADDED COPPER	GAIN	FEED CONVERSION	ALKALINE PHOSPHATASE LEVELS*
(PPM)	(GRAMS)		
0	290	1.78	87.0000
10	299	1.71	89.4777
20	302	1.73	89.9864
30	308	1.76	93.1176
40	317	1.77	99.4820
60	299	1.78	93.2400
80	299	1.78	93.2400

* ALKALINE PHOSPHATASE ACTIVITIES ARE EXPRESSED AS MILLIGRAMS PHOSPHORUS PER GRAM KIDNEY TISSUE (WET WEIGHT)

GROUPS OF 10 CHICKS EACH WERE USED IN THIS STUDY.

THE RATE OF GAIN, FEED CONVERSION AND ALKALINE PHOSPHATASE ACTIVITIES ARE PRESENTED IN TABLE LV.

THE RATE OF GAIN OF THE CHICKS WAS SUBJECTED TO A STATISTICAL ANALYSIS OF VARIANCE, WHICH IS PRESENTED IN TABLE LVI. SIGNIFICANT DIFFERENCES BETWEEN THE RATES OF GAIN WERE FOUND. THE BASAL RATION CONTAINING SUPPLEMENTAL LEVELS OF 10 PPM MOLYBDENUM AND ONE PPM TUNGSTEN WAS COMPARED TO THE SAME RATION WITH THE VARIOUS LEVELS OF COPPER SUPPLEMENTATIONS. IT WAS FOUND THAT THE ADDITION OF 10 PPM COPPER GAVE A SLIGHT STIMULATION IN GROWTH. THIS WAS MAGNIFIED

TABLE LVI

ANALYSIS OF VARIANCE ON RATE OF GAIN OF VANTRESS-WPR CHICKS FED A PRACTICAL BROILER RATION WITH COPPER SUPPLEMENTATION LEVELS

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	139		
BETWEEN COPPER TREATMENTS	6	1669	3.43**
BASAL VS 10 PPM COPPER	1	731	1.50
BASAL VS 20 PPM COPPER	1	1222	2.50
BASAL VS 30 PPM COPPER	1	2739	5.63*
BASAL VS 40 PPM COPPER	1	6003	12.32**
BASAL VS 60 PPM COPPER	1	837	1.72
BASAL VS 80 PPM COPPER	1	440	0.90
ERROR	133	487	

* SIGNIFICANT AT 0.05 LEVEL OF PROBABILITY
** SIGNIFICANT AT 0.01 LEVEL OF PROBABILITY

SOMEWHAT UPON THE ADDITION OF 20 PPM COPPER SO THAT THE INCREASE IN GAIN APPROACHED SIGNIFICANCE. THE SUPPLEMENTATION OF 30 PPM COPPER RESULTED IN A SIGNIFICANT STIMULATION IN THE RATE OF GAIN, WHILE THE ADDITION OF 40 PPM COPPER TO THIS RATION RESULTED IN A HIGHLY SIGNIFICANT INCREASE IN THE RATE OF GAIN. HIGHER SUPPLEMENTATIONS OF COPPER (60 AND 80 PPM) DID NOT PROMOTE ANY GREATER RATE OF GAIN THAN WAS OBTAINED FROM THE BASAL RATION.

ATTENTION IS CALLED TO THE FACT THAT THE BEST RATE OF GAIN IN THIS TRIAL WAS ATTAINED WHEN 40 PPM COPPER WAS ADDED TO THE PRACTICAL BROILER RATION WHILE THE ADDITION OF 30 PPM GAVE BEST RESULTS IN THE

CASE OF THE PURIFIED RATION. HOWEVER, AS REPORTED EARLIER, CHEMICAL ANALYSIS OF THE PURIFIED DIET SHOWED THAT THIS DIET CONTAINED 25.85 PPM COPPER, WHILE THE PRACTICAL BROILER RATION CONTAINED 10.80 PPM. THE TOTAL INTAKE OF COPPER WAS NOT AS DIFFERENT THEN AS IT MIGHT APPEAR, THE VALUES BEING 55.85 PPM FOR THE PURIFIED DIET AND 50.80 PPM IN THE CASE OF THE PRACTICAL BROILER RATION.

AN ANALYSIS OF VARIANCE WAS MADE ON THE FEED CONVERSION DATA OBTAINED IN THIS PORTION OF THE STUDY. THE ANALYSIS IS PRESENTED IN TABLE LVII. NO STATISTICALLY SIGNIFICANT DIFFERENCES WERE FOUND TO EXIST IN THESE DATA.

THE ALKALINE PHOSPHATASE LEVELS REMAINED MUCH MORE CONSTANT WITH THE VANTRESS-WPR CHICKS ON THE PRACTICAL BROILER RATION THAN IN THE CASE OF THE CHICKS FROM THE TUNGSTATE SUPPLEMENTED HENS FED THE PURIFIED RATION. THE ENZYME VALUES FROM THE CHICKS IN THIS PORTION OF THE STUDY MORE CLOSELY APPROACHED THE RELATIVE CHANGES THAT WERE FOUND IN THE CASE OF THE CHICKS, FROM THE NON-SUPPLEMENTED HENS, FED THE PURIFIED DIET. AT THE 10 AND 20 PPM LEVELS OF COPPER SUPPLEMENTATION THE ENZYME ACTIVITY WAS ABOUT THE SAME AS THAT FOUND TO EXIST IN CHICKS FED THE BASAL RATION. THE ADDITION OF 30 PPM COPPER TO THE BASAL RATION CAUSED AN INCREASE OF SEVEN PER CENT IN THE ALKALINE PHOSPHATASE ACTIVITY, WHICH INCREASE WAS APPROXIMATELY DOUBLED UPON THE ADDITION OF 40 PPM COPPER. IT WAS INTERESTING TO NOTE THAT THE ADDITION OF 60 PPM COPPER TO THE BASAL RATION HAD THE SAME EFFECT ON THE ENZYME ACTIVITY AS DID THE 30 PPM ADDITIONAL COPPER, ALTHOUGH SAMPLING ERROR MIGHT BE RESPONSIBLE FOR THIS OBSERVATION. AN INCREASE OF APPROXIMATELY 16 PER CENT WAS FOUND WHEN THE BASAL RATION WAS SUPPLEMENTED WITH 80 PPM COPPER.

TABLE LVII

ANALYSIS OF VARIANCE ON FEED CONVERSION DATA OBTAINED FROM VANTRESS-WPR CHICKS FED PRACTICAL BROILER RATION PLUS VARIOUS LEVELS OF COPPER SUPPLEMENTATION

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	13		
BETWEEN COPPER LEVELS	6	91.66	1.31
ERROR	7	60.00	

IT IS OF INTEREST TO NOTE THE FACT THAT THE VALUES OBTAINED FROM THE CHICKS FED THE PRACTICAL RATION APPEAR TO BE HIGHER THAN THE ALKALINE PHOSPHATASE VALUES OBTAINED FROM THE CHICKS FED THE PURIFIED DIET. THIS APPEARS TO FURTHER SUBSTANTIATE THE POSTULATE THAT ALKALINE PHOSPHATASE APPEARS TO BE DIRECTLY OR INDIRECTLY RELATED TO COPPER AND TUNGSTEN METABOLISM.

SUMMARY

IT WAS FOUND THAT ALMOST IDENTICAL GROWTH STIMULATION RESULTED FROM THE SUPPLEMENTATION OF COPPER TO THE PURIFIED DIET REGARDLESS OF THE DAM TREATMENT. THE ADDITION OF 10 PPM COPPER CAUSED A SIGNIFICANT INCREASE IN THE RATE OF GAIN, WHILE SUPPLEMENTATIONS OF 20 AND 30 PPM COPPER RESULTED IN HIGHLY SIGNIFICANT GAINS, WITH THE HIGHER LEVEL OF COPPER SUPPORTING THE BETTER GAINS, OVER THAT OBTAINED WITH THE BASAL RATION. RESULTS WITH THE COMMERCIAL BROILER CHICKS FED THE PRACTICAL BROILER RATION WERE SIMILAR BUT HIGHER LEVELS OF COPPER WERE NEEDED TO GIVE GAINS THAT DIFFERED SIGNIFICANTLY FROM THAT OBTAINED ON THE BASAL RATION. THE ADDITION OF 40 PPM COPPER RESULTED IN SIGNIFICANT

INCREASES IN GAINS ON THIS RATION. SINCE THE PRACTICAL BROILER RATION WAS FOUND TO CONTAIN 15 PPM LESS COPPER THAN THE PURIFIED DIETS, THE LEVELS OF COPPER THAT GAVE THE GREATEST INCREASES IN GAIN VARIED ABOUT FIVE PPM IN THE LEVEL OF TOTAL COPPER CONTENT.

THE ALKALINE PHOSPHATASE VALUES SHOWED A GREAT DEAL OF SIMILARITY BETWEEN THE CHICKS FROM NON-SUPPLEMENTED DAMS, FED THE PURIFIED DIET AND THE COMMERCIAL BROILER CHICKS FED THE PRACTICAL BROILER RATION. AS THE LEVEL OF COPPER WAS INCREASED, THE LEVEL OF ENZYME ACTIVITY STEADILY INCREASED. THE ADDITION OF 40 PPM COPPER CAUSED AN INCREASED ENZYME ACTIVITY AMOUNTING TO ABOUT 30 PER CENT. THE ENZYME LEVEL OF THE CHICKS FROM TUNGSTATE SUPPLEMENTED DAMS WAS QUITE DIFFERENT. THE SUPPLEMENTATION OF DIFFERING LEVELS OF COPPER CAUSED DRAMATIC INCREASES IN THE LEVEL OF THIS ENZYME. THE ADDITION OF 40 PPM COPPER TO THE PURIFIED DIET CAUSED AN INCREASED ALKALINE PHOSPHATASE ACTIVITY OF 80 PER CENT. THIS FACT GAVE RISE TO THE POSTULATION THAT THE SUPPLEMENTAL COPPER MIGHT HAVE BEEN OVERCOMING A TUNGSTATE TOXICITY, CARRIED-OVER FROM THE DAM, AND THIS INCREASED ENZYME ACTIVITY HAD A DIRECT OR INDIRECT CONNECTION WITH SOME METABOLIC FUNCTION. DATA WHICH SUPPORT SUCH A POSTULATE WERE THAT THERE APPEARED TO BE NO DIFFERENCES EXISTING BETWEEN THE GROUPS OF CHICKS FROM THE TUNGSTATE SUPPLEMENTED DAMS AND THE CHICKS FROM THE NON-SUPPLEMENTED DAMS. THIS STUDY MARKED THE FIRST TIME THAT THIS HAD BEEN FOUND TO BE TRUE.

F. Sulfate-Sulfur Supplementation of Chicks Diets

Since various workers had suggested that sulfate sulfur was interrelated with molybdenum, with copper, and with both of these elements, it seemed appropriated to undertake a study to investigate the effect of various inorganic sulfur additions to a ration containing the most desirable levels of molybdenum, tungsten, and copper. The sulfur was added in the form of anhydrous sodium sulfate. The levels of molybdenum, tungsten, and copper were those that had given the maximum growth response in the previous studies. The purified basal ration was supplemented with 10 ppm molybdenum, one ppm tungsten, and 30 ppm copper. The practical broiler ration was supplemented with the same levels of molybdenum and tungsten but 40 ppm copper was added since this level had given maximum growth with this particular ration. It should be recognized that these two additions of copper yielded essentially the same total copper content for the two rations. The sulfate sulfur additions were made to the two basal rations mentioned above. Levels of supplementation were 0, 1000, 2000, 3000, and 4000 ppm sulfur. The purified ration contained 11,649 ppm total sulfur and the practical ration contained 4635 ppm. The higher sulfur content was partially due to the addition of one per cent methionine to the purified ration.

Criteria used in this study were the rates of gain and feed conversions. Alkaline phosphatase determinations on kidney tissue homogenates were made at the end of the 20-day growth period for these chicks.

TABLE LVIII

RESULTS OF GAIN, FEED CONVERSIONS, AND ALKALINE PHOSPHATASE ACTIVITIES OF CHICKS, FROM TUNGSTATE SUPPLEMENTED DAMS, FED PURIFIED DIET WITH SULFATE SUPPLEMENTATION

ADDED SULFUR (PPM)	GAIN (GRAMS)	FEED CONVERSION	ALKALINE PHOSPHATASE LEVELS*
0	249	1.42	42.4447
1000	271	1.43	45.7791
2000	268	1.40	42.8750
3000	263	1.44	71.5447
4000	257	1.52	75.5290

* ALKALINE PHOSPHATASE ACTIVITY IS EXPRESSED AS MILLIGRAMS PHOSPHORUS PER GRAM KIDNEY TISSUE (WET WEIGHT)

1. PURIFIED RATION STUDIES WITH CHICKS FROM TUNGSTATE SUPPLEMENTED DAMS

THE CHICKS FOR THIS STUDY CAME FROM DAMS THAT HAD BEEN FED THE BREEDER RATION CONTAINING 500 PPM SUPPLEMENTAL TUNGSTATE. THE RATE OF GAIN, FEED CONVERSIONS, AND ALKALINE PHOSPHATASE ACTIVITIES FOR THIS STUDY ARE PRESENTED IN TABLE LVIII. STATISTICAL ANALYSIS OF THE RATE OF GAIN WERE MADE AND ARE SHOWN IN TABLE LIX. WHEN THE GAIN OBTAINED FROM THE BASAL RATION WAS COMPARED TO THE VARIOUS LEVELS OF SULFUR SUPPLEMENTATION IT WAS FOUND THAT THE ADDITION OF 1000 AND 2000 PPM SULFUR AS SULFATE CAUSED HIGHLY SIGNIFICANT INCREASES IN THE RATE OF

TABLE LIX

ANALYSIS OF VARIANCE ON GAIN OF CHICKS, FROM TUNGSTATE SUPPLEMENTED DAMS, FED A PURIFIED DIET WITH SULFATE SUPPLEMENTATIONS

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	198		
BETWEEN SULFUR LEVELS	4	1595	3.05*
BASAL VS 1000 PPM SULFUR	1	5153	9.85**
BASAL VS 2000 PPM SULFUR	1	3842	7.34**
BASAL VS 3000 PPM SULFUR	1	2146	4.10*
BASAL VS 4000 PPM SULFUR	1	960	1.83
ERROR	194	523	

* SIGNIFICANT AT 0.05 LEVEL OF PROBABILITY

** SIGNIFICANT AT 0.01 LEVEL OF PROBABILITY

GAIN, WITH THE LOWER LEVEL OF SUPPLEMENTATION RESULTING IN THE GREATER GROWTH STIMULATION. THE ADDITION OF 3000 PPM SULFATE SULFUR TO THIS PURIFIED RATION GAVE A SIGNIFICANT STIMULATION IN THE RATE OF GAIN COMPARED TO THAT ATTAINED BY THE BASAL RATION. THE HIGHEST LEVEL OF SUPPLEMENTAL SULFUR TENDED TO GIVE A SLIGHT INCREASE IN THE RATE OF GAIN BUT THIS INCREASE WAS FOUND NOT TO BE STATISTICALLY SIGNIFICANT.

A STATISTICAL ANALYSIS REVEALED THAT A HIGHLY SIGNIFICANT DIFFERENCE EXISTED IN THE FEED CONVERSIONS BETWEEN THE LEVELS OF SUPPLEMENTAL SULFUR (TABLE LX). WHEN THE FEED CONVERSION DATA FROM THE VARIOUS LOTS RECEIVING THE SUPPLEMENTAL LEVELS OF SULFUR WERE STATISTICALLY COMPARED TO THAT ATTAINED FROM THE BASAL RATION IT WAS FOUND THAT THE ADDITIONS

TABLE LX

ANALYSIS OF VARIANCE ON FEED CONVERSIONS OF CHICKS, FROM TUNGSTATE SUPPLEMENTED DAMS, FED A PURIFIED DIET WITH SULFATE SUPPLEMENTATIONS

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	19		
BETWEEN SULFUR LEVELS	4	77.25	8.52*
BASAL VS 1000 PPM SULFUR	1	2.00	0.23
BASAL VS 2000 PPM SULFUR	1	16.00	1.87
BASAL VS 3000 PPM SULFUR	1	8.00	0.94
BASAL VS 4000 PPM SULFUR	1	181.00	21.22**
ERROR	15	9.00	

* SIGNIFICANT AT 0.05 LEVEL OF PROBABILITY

** SIGNIFICANT AT 0.01 LEVEL OF PROBABILITY

OF 1000, 2000 AND 3000 PPM SULFUR DID NOT PRODUCE RESULTS THAT DIFFERED FROM THAT OF THE BASAL RATION. HOWEVER, WHEN THE RATION WAS SUPPLEMENTED WITH 4000 PPM SULFUR A HIGHLY SIGNIFICANT DEPPESSION OF THE FEED CONVERSION BELOW THAT OF THE UNSUPPLEMENTED BASAL RATION WAS FOUND. THE ALKALINE PHOSPHATASE LEVELS OF THE CHICKS FED THE 1000 AND 2000 PPM SULFATE WERE QUITE SIMILAR TO THAT OF THE CHICKS FED THE BASAL RATION. WHEN THE RATION WAS SUPPLEMENTED WITH 3000 PPM SULFATE SULFUR THERE WAS A SHARP INCREASE OF ALMOST 70 PER CENT IN THE ENZYME ACTIVITY OVER THAT OBTAINED FOR THE BASAL RATION. THE SUPPLEMENTATION OF THE BASAL RATION WITH 4000 PPM SULFUR RESULTED IN A STILL HIGHER RISE IN THE ENZYME CONCENTRATION AMOUNTING TO ALMOST 78 PER CENT OVER THE VALUE FOR THE BASAL RATION.

TABLE LXI

RESULTS OF RATE OF GAIN, FEED CONVERSION, AND ALKALINE PHOSPHATASE ACTIVITIES OF CHICKS, FROM NON-SUPPLEMENTED DAMS, FED A PURIFIED RATION WITH SULFATE SUPPLEMENTATIONS

ADDED SULFUR	GAIN	FEED CONVERSION	ALKALINE PHOSPHATASE ACTIVITY*
(PPM)	(GRAMS)		
0	264	1.42	40.8790
1000	278	1.39	40.3209
2000	276	1.42	39.0239
3000	262	1.48	51.6558
4000	260	1.57	51.0267

* ALKALINE PHOSPHATASE ACTIVITY EXPRESSED AS MILLIGRAMS PHOSPHORUS PER GRAM KIDNEY TISSUE (WET WEIGHT).

II. PURIFIED RATION STUDIES WITH CHICKS FROM NON-SUPPLEMENTED DAMS

THE SECOND PORTION OF THIS STUDY WAS CONCERNED WITH THE EFFECTS OF SUPPLEMENTAL SULFUR WHEN ADDED TO A PURIFIED DIET FED TO CHICKS FROM NON-SUPPLEMENTED WHITE PLYMOUTH ROCK HENS. THE PRIMARY DIFFERENCE BETWEEN THIS SECTION AND THE PREVIOUS SECTION WAS THE DIFFERENCE IN THE DAM TREATMENT. THE SAME CRITERIA AS DESCRIBED IN THE PREVIOUS SECTION WERE USED IN THIS STUDY.

THE DATA FROM THIS TRIAL ARE PRESENTED IN TABLE LXI. THE RESULTS OF A STATISTICAL ANALYSIS OF VARIANCE ON THE RATE OF GAIN IS PRESENTED IN TABLE LXII. AS CAN BE SEEN FROM THIS ANALYSIS OF VARIANCE, A SIGNIFICANT DIFFERENCE WAS FOUND TO EXIST BETWEEN THE VARIOUS LEVELS

TABLE LXII

RESULTS OF ANALYSIS OF VARIANCE ON GAIN OF CHICKS, FROM NON-SUPPLEMENTED DAMS, FED A PURIFIED DIET WITH VARIOUS LEVELS OF SUPPLEMENTAL SULFATE

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	199	-	-
BETWEEN SULFUR LEVELS	4	1497	3.06*
BASAL VS 1000 PPM SULFUR	1	2161	4.42*
BASAL VS 2000 PPM SULFUR	1	1716	3.53
BASAL VS 3000 PPM SULFUR	1	123	0.25
BASAL VS 4000 PPM SULFUR	1	26	0.05
ERROR	195	489	-

* SIGNIFICANT AT 0.05 LEVEL OF PROBABILITY

OF SULFUR ADDED TO THE PURIFIED DIET. WHEN THE BASAL RATION WAS COMPARED TO THE VARIOUS LEVELS OF SUPPLEMENTAL SULFATE SULFUR IT WAS FOUND THAT THE ADDITION OF 1000 PPM INORGANIC SULFUR CAUSED A SIGNIFICANT INCREASE IN THE 20-DAY GAIN OF THESE CHICKS. THE INCLUSION OF 2000 PPM SULFUR TO THE PURIFIED DIET GAVE AN INCREASE IN GAIN THAT APPROACHED SIGNIFICANCE BUT WAS SLIGHTLY UNDER THAT CAUSED BY THE LOWER LEVEL OF SUPPLEMENTATION. THE SUPPLEMENTATION OF 3000 AND 4000 PPM SULFUR WERE WITHOUT EFFECT WHEN COMPARED TO THE GAIN ATTAINED BY THE BASAL PURIFIED DIET. THESE RESULTS DID NOT COMPLETELY AGREE WITH THOSE OBTAINED FROM THE CHICKS FROM TUNGSTATE SUPPLEMENTED DAMS WHERE ALL LEVELS OF SULFATE SULFUR SUPPLEMENTATION RESULTED IN STATISTICALLY SIGNIFICANT INCREASES IN THE RATE OF GAIN. HOWEVER, THE SAME LEVEL

TABLE LXIII

RESULTS OF ANALYSIS OF VARIANCE ON FEED CONVERSIONS OBTAINED ON CHICKS, FROM NON-SUPPLEMENTED DAMS, FED A PURIFIED RATION WITH VARIOUS SULFATE SUPPLEMENTATIONS

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	19		
BETWEEN SULFUR LEVELS	4	254.3	5.70**
BASAL VS 1000 PPM SULFUR	1	16.0	0.36
BASAL VS 2000 PPM SULFUR	1	2.0	0.04
BASAL VS 3000 PPM SULFUR	1	36.0	0.81
BASAL VS 4000 PPM SULFUR	1	338.0	7.57*
ERROR	15	44.6	

* SIGNIFICANT AT 0.05 LEVEL OF PROBABILITY

(1000 PPM) IN BOTH CASES GAVE THE GREATEST INCREASE IN GAIN OVER THAT FOR THE BASAL RATION.

A STATISTICAL ANALYSIS OF VARIANCE WAS MADE ON THE FEED CONVERSION DATA AND IS PRESENTED IN TABLE LXIII. STATISTICAL DIFFERENCES WERE FOUND TO EXIST BETWEEN THE GROUPS FED THE VARIOUS LEVELS OF SUPPLEMENTAL SULFUR. IT WAS FOUND BY STATISTICAL COMPARISONS THAT THE LOWER THREE LEVELS OF SULFUR SUPPLEMENTATIONS (1000, 2000, AND 3000 PPM) WERE WITHOUT EFFECT COMPARED TO THE BASAL RATION. THE INCLUSION OF 4000 PPM SULFUR IN THE PURIFIED DIET CAUSED A SIGNIFICANT DEPRESSION IN THE FEED CONVERSION RATE. THIS WAS THE SAME EFFECT NOTED WHEN THIS RATION WAS FED TO THE CHICKS FROM TUNGSTATE SUPPLEMENTED HENS, AS REPORTED IN

THE PREVIOUS SECTION.

THE ALKALINE PHOSPHATASE VALUES FOR THE BASAL DIET AND THE 1000 AND 2000 PPM LEVELS OF SUPPLEMENTATION WERE QUITE SIMILAR. THE 3000 PPM AND 4000 PPM ADDITIONS OF SULFUR RESULTED IN AN APPARENT INCREASE OF 26 PER CENT IN THE ALKALINE PHOSPHATASE VALUES.

III. PRACTICAL RATION STUDIES WITH VANTRESS -WPR CHICKS

THE SAME CRITERIA REPORTED EARLIER IN THIS STUDY WERE USED TO DETERMINE WHAT EFFECT THE SUPPLEMENTAL LEVELS OF INORGANIC SULFUR MIGHT HAVE ON COMMERCIAL BROILER CHICKS FED A PRACTICAL CORN-SOYBEAN MEAL BROILER RATION. THE GAINS FOR A 20-DAY PERIOD, FEED CONVERSIONS, AND ALKALINE PHOSPHATASE ACTIVIES WERE DETERMINED AND ARE PRESENTED IN TABLE LXIV.

TABLE LXIV

RATE OF GAIN, FEED CONVERSION DATA AND ALKALINE PHOSPHATASE DETERMINATIONS ON VANTRESS-WPR CHICKS FED A PRACTICAL BROILER RATION WITH SULFATE SUPPLEMENTS

ADDED SULFUR (PPM)	GAIN (GRAMS)	FEED CONVERSION	ALKALINE PHOSPHATASE ACTIVITY*
0	298	1.72	95.4942
1000	318	1.70	92.8073
2000	308	1.67	100.6200
3000	300	1.74	117.9846
4000	303	1.78	123.7832

* ALAKLINE PHOSPHATASE ACTIVITY EXPRESSED AS MILLIGRAMS PHOSPHORUS PER GRAM KIDNEY TISSUE (WET WEIGHT).

TABLE LXV

RESULTS OF ANALYSIS OF VARIANCE ON RATE OF GAIN ON VANTRESS-WPR CHICKS FED A PRACTICAL BROILER RATION WITH SUPPLEMENTAL LEVELS OF SULFATE

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	99		
BETWEEN SULFUR LEVELS	4	1424.5	3.07*
BASAL VS 1000 PPM SULFUR	1	4041.0	8.13**
BASAL VS 2000 PPM SULFUR	1	1092.0	2.19
BASAL VS 3000 PPM SULFUR	1	57.0	0.11
BASAL VS 4000 PPM SULFUR	1	1250.0	2.52
ERROR	95	497.5	

* SIGNIFICANT AT 0.05 LEVEL OF PROBABILITY

** SIGNIFICANT AT 0.01 LEVEL OF PROBABILITY

A STATISTICAL ANALYSIS WAS MADE ON THE RATE OF GAIN OBTAINED IN THIS STUDY. THE ANALYSIS OF VARIANCE IS PRESENTED IN TABLE LXV. IT WAS FOUND THAT SIGNIFICANT DIFFERENCES EXISTED BETWEEN GROUPS FED DIFFERENT LEVELS OF SULFATE SULFUR. UPON COMPARING THE VARIOUS GROUPS WITH THE BASAL RATION IT WAS FOUND THAT THE INCLUSION OF 1000 PPM INORGANIC SULFUR IN THE PRACTICAL BROILER RATION RESULTED IN A STATISTICALLY SIGNIFICANT IMPROVEMENT IN THE RATE OF GAIN. THE RESULTS OBTAINED FROM OTHER GROUPS FED VARIOUS LEVELS OF INORGANIC SULFUR WERE FOUND TO BE NOT STATISTICALLY SIGNIFICANT ALTHOUGH SOME DEGREE OF IMPROVEMENT IN GAIN WAS OBSERVED. THIS WAS THE SAME PATTERN THAT WAS FOUND WHEN THE NON-SUPPLEMENTED WHITE PLYMOUTH ROCK CHICKS WERE FED THE

TABLE LXVI

RESULTS OF ANALYSIS OF VARIANCE ON FEED CONVERSIONS OBTAINED FROM VANTRESS-WPR CHICKS FED A PRACTICAL BROILER RATION WITH VARIOUS LEVELS OF SULFATE

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	9		
BETWEEN SULFUR LEVELS	4	257.0	1.67
ERROR	5	154.0	

PURIFIED DIET.

THE RESULTS OF A STATISTICAL ANALYSIS ON THE FEED CONVERSION DATA ARE PRESENTED IN TABLE LXVI. NO REAL DIFFERENCES WERE FOUND TO EXIST BETWEEN THE FEED CONVERSION RATES. SOME DIFFERENCES WERE APPARENT BUT DUE TO THE SMALL NUMBER OF OBSERVATIONS AND THE VARIATIONS BETWEEN REPLICATE GROUPS THESE DIFFERENCES WERE NOT STATISTICALLY SIGNIFICANT.

THE KIDNEY ALKALINE PHOSPHATASE LEVELS TENDED TO REMAIN RELATIVELY CONSTANT WHEN SULFATE SULFUR WAS ADDED TO THE RATION AT THE LEVELS OF 1000 AND 2000 PPM. IN THE CASE OF THE CASE OF THE VANTRESS-WPR CHICKS FED THE PRACTICAL BROILER RATION, THE ADDITION OF 3000 PPM OF SULFATE SULFUR RESULTED IN AN INCREASE OF ALMOST 24 PER CENT COMPARED TO THE VALUE OBTAINED FROM THE BASAL RATION. THE INCLUSION OF 4000 PPM SULFATE SULFUR IN THE PRACTICAL BROILER RATION RESULTED IN AN INCREASED ENZYME LEVEL OF APPROXIMATELY 30 PER CENT.

Summary

The addition of 1000 and 2000 ppm of sulfate sulfur to a purified diet to which had been added 10 ppm molybdenum, one ppm tungsten, and 30 ppm copper resulted in a significant increase in rates of gain for chicks regardless of whether the dams received tungstate supplementations. In the case of the chicks from hens fed the tungstate supplemented ration, the addition of 3000 ppm sulfur resulted in a significant increase in gain over that obtained on the basal ration. The gain was essentially the same as that for the chicks from the unsupplemented breeder hens, which in turn was not significantly different from that of the basal fed groups. This significant increase is due primarily to the poorer gains experienced by the basal ration-fed chicks from the tungstate supplemented breeder hens. The basal-ration-fed chicks from the unsupplemented breeder hens grew at a significantly greater rate than did those from the tungsten supplemented hens. This observation is difficult to explain unless the tungsten carry-over from the hens to the chicks is depressing growth and that this depression is overcome by the addition of sulfate. This condition is essentially the same as that observed in the molybdenum and tungsten studies where it was suspected that the result was due to the tungsten carry-over in the chicks. That sulfate supplementation helped counteract this carry-over effect would only partially explain this effect since significant increases in gains were also experienced due to sulfate additions to the rations of chicks whose dams had not received any tungsten supplementation. This was true for both the White Plymouth Rock chicks and the commercial chicks. Increasing the

LEVEL OF SULFATE SUPPLEMENTATION ABOVE 1000 PPM APPEARED TO REDUCE THE RATE OF GAIN IN ALL CASES. HOWEVER THIS REDUCTION WAS NOT OF SUFFICIENT MAGNITUDE TO BE STATISTICALLY SIGNIFICANT. THIS LEVEL OF SUPPLEMENTATION RESULTED IN A TOTAL SULFUR CONTENT OF 12649 PPM IN THE CASE OF THE PURIFIED RATION AND 5635 PPM IN THE CASE OF THE PRACTICAL RATION. EVEN THOUGH ONE PER CENT METHIONINE WAS ADDED TO THE PURIFIED RATION, THIS WOULD ACCOUNT FOR ONLY 2300 PPM SULFUR AS METHIONINE AND THE COMPARATIVE LEVELS OF THE TWO RATIONS WERE STILL RADICALLY DIFFERENT. THE RESULTS WOULD INDICATE THAT THE FORMS OF THE SULFUR IN THE TWO RATIONS WERE QUITE DIFFERENT AND THAT THE SULFATE ADDITIONS WERE FUNCTIONING PRIMARILY AS SULFATE AND INDEPENDENTLY OF THE REMAINING SULFUR CONTAINING COMPOUNDS IN THE RATION. THE RESULTS OF THIS STUDY SUPPORT THE SUGGESTION OF GORDON (1957) AND MACHLIN (1955) THAT THERE APPEARS TO BE A REQUIREMENT FOR SULFATE BY THE CHICK UNDER CERTAIN CONDITIONS. THIS DATA ALSO SUGGESTS AN ASSOCIATION BETWEEN TUNGSTEN AND SULFATE IN THE DIETS OF GROWING CHICKS.

SULFATE ADDITION DID NOT AFFECT FEED CONVERSIONS EXCEPT AT THE 4000 PPM ADDITIONS TO THE PURIFIED RATION. THE ALKALINE PHOSPHATASE VALUES FOR CHICKS FED THE PURIFIED RATIONS WITH 1000 AND 2000 PPM SULFATE ADDITIONS WERE QUITE SIMILAR; HOWEVER, THERE WAS A DRAMATIC INCREASE (70 AND 78 PER CENT) IN THE ALKALINE PHOSPHATASE VALUES FOR THE 3000 AND 4000 PPM ADDITIONS TO THE PURIFIED DIETS OF CHICKS FROM TUNGSTATE FED DAMS. THE INCREASE IN THIS ENZYME FOR CHICKS FROM NON-SUPPLEMENTED DAMS WAS NOT SO DRAMATIC, BEING ONLY 26 PER CENT AT THE HIGHER LEVELS OF SULFATE ADDITION. THE ALKALINE PHOSPHATASE VALUES FOR THE COMMERCIAL BROILER CHICKS FED THE PRACTICAL RATION WITH 1000 AND

2000 PPM SULFATE WERE CONSIDERABLY HIGHER THAN FOR CHICKS FED THE PURIFIED RATIONS, BUT DID NOT DIFFER SIGNIFICANTLY AMONG THEMSELVES. THE ADDITION OF 3000 AND 4000 PPM SULFATE RESULTED IN INCREASES OF 24 PER CENT AND 30 PER CENT, RESPECTIVELY. THESE INCREASES ARE QUITE SIMILAR IN MAGNITUDE IN COMPARISON TO THOSE OF THE CHICKS FROM THE NON-SUPPLEMENTED DAMS FED THE PURIFIED RATION. AS WAS THE CASE WITH COPPER, THE ALKALINE PHOSPHATASE LEVELS WERE QUITE DIFFERENT IN THE CASE OF THE CHICKS FROM THE TUNGSTATE SUPPLEMENTED DAMS.

IT IS NOT FELT THAT SULFATE ADDITION IS DIRECTLY RELATED TO THE ALKALINE PHOSPHATASE VALUES, BUT RATHER THE ALKALINE PHOSPHATASE INCREASES DUE TO CERTAIN STRESS CONDITIONS BROUGHT ABOUT BY THE INCREASED SUPPLEMENTATIONS OF THESE MINERALS.

AGAIN THERE APPEARED TO BE NO ASSOCIATION BETWEEN GROWTH RATE AND THE KIDNEY ALKALINE PHOSPHATASE ACTIVITIES.

G. Phosphorus Supplementation of Chick Diets

Since copper and sulfate supplementations had resulted in dramatic alkaline phosphatase increases in kidney tissue with high levels of these elements, it appeared appropriate to study different levels of phosphorus in the rations.

Both the purified diet and the practical broiler ration to which had been added ten ppm molybdenum, one ppm tungsten, 30 or 40 ppm copper (to the purified diet and practical ration, respectively), and 1000 ppm inorganic sulfate were supplemented with various levels of phosphorus. The levels of phosphorus supplementations were 0, 500, 1000, 1500, and 2000 ppm. The phosphorus was added as ortho-phosphoric acid. Exactly the same management procedures were followed as were practiced in earlier studies.

Twenty-day weight gains and feed conversions were the major criteria used in this study, although alkaline phosphatase determinations were made on kidney tissue homogenates at the conclusion of each trial.

1. Purified Ration Studies With Chicks From Tungstate Supplemented Dams

Two hundred chicks, brooded in 20 groups of 10 chicks each were used in this portion of the study. The gains, feed conversions, and alkaline phosphatase activities obtained are presented in Table LXVII.

An analysis of variance on the rate of gains from these White Plymouth Rock chicks is presented in Table LXVIII, and revealed that there were no real differences in the rates of gain among the various

TABLE LXVII

RESULTS OF GAIN, FEED CONVERSIONS, AND ALKALINE PHOSPHATASE VALUES ON CHICKS, FROM TUNGSTATE SUPPLEMENTED DAMS, FED A PURIFIED RATION PLUS SUPPLEMENTAL LEVELS OF PHOSPHORUS

ADDED PHOSPHORUS (PPM)	GAIN (GRAMS)	FEED CONVERSION	ALKALINE PHOSPHATASE ACTIVITY*
0	262	1.47	44.4949
500	260	1.48	47.1515
1000	265	1.47	45.8077
1500	254	1.48	56.7633
2000	255	1.58	55.5871

* ALKALINE PHOSPHATASE ACTIVITY IS EXPRESSED AS MILLIGRAMS PHOSPHORUS PER GRAM KIDNEY TISSUE (WET WEIGHT).

TABLE LXVIII

RESULTS OF ANALYSIS OF VARIANCE ON GAIN OF CHICKS, FROM TUNGSTATE SUPPLEMENTED DAMS, FED PURIFIED DIET WITH SUPPLEMENTAL LEVELS OF PHOSPHORUS

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	199		
BETWEEN PHOSPHORUS LEVELS	4	1178	1.12
ERROR	195	1049	

TABLE LXIX

ANALYSIS OF VARIANCE ON FEED CONVERSIONS OF CHICKS, FROM TUNGSTATE FED DAMS, FED A PURIFIED RATION WITH SUPPLEMENTAL LEVELS OF PHOSPHORUS

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	19		
BETWEEN PHOSPHORUS LEVELS	4	96.2	1.47
ERROR	15	65.3	

GROUPS IN THIS STUDY.

A STATISTICAL ANALYSIS OF VARIANCE WAS MADE ON THE FEED CONVERSION DATA IS PRESENTED IN TABLE LXVIX. IT WAS FOUND THAT THERE WERE NO REAL DIFFERENCES EXISTING AMONG THE VARIOUS GROUPS DURING THIS 20-DAY TRIAL PERIOD.

THE LEVEL OF ALKALINE PHOSPHATASE ACTIVITY REMAINED RELATIVELY STABLE WHEN PHOSPHORUS WAS ADDED TO THE BASAL RATION AT THE LEVELS OF 500 AND 1000 PPM. THE ADDITIONS OF 1500 AND 2000 PPM PHOSPHORUS TENDED TO CAUSE A DEPRESSION IN THE RATE OF GAIN WHICH WAS NOT SIGNIFICANT; HOWEVER, ALKALINE PHOSPHATASE ACTIVITIES FOR THESE LEVELS OF PHOSPHORUS SUPPLEMENTATION WAS INCREASED SLIGHTLY MORE THAN 25 PER CENT.

II. Purified Ration Studies With Chicks From Non-Supplemented Dams

THE SAME EXPERIMENTAL DESIGN AS DESCRIBED IN THE PREVIOUS SECTION

TABLE LXX

RESULTS OF GAIN, FEED CONVERSIONS, AND ALKALINE PHOSPHATASE ACTIVITIES ON CHICKS, FROM NON-SUPPLEMENTED DAMS, FED PURIFIED DIET WITH PHOSPHORUS SUPPLEMENTATIONS

ADDED PHOSPHORUS (PPM)	GAIN (GRAMS)	FEED CONVERSION	ALKALINE PHOSPHATASE ACTIVITY*
0	263	1.45	50.7994
500	259	1.48	52.6927
1000	255	1.50	52.2052
1500	257	1.48	56.5277
2000	255	1.51	55.9670

* ALKALINE PHOSPHATASE ACTIVITY IS EXPRESSED AS MILLIGRAMS PHOSPHORUS PER GRAM KIDNEY TISSUE (WET WEIGHT).

TABLE LXXI

ANALYSIS OF VARIANCE ON GAIN OF CHICKS, FROM NON-SUPPLEMENTED DAMS, FED PURIFIED DIETS WITH PHOSPHORUS SUPPLEMENTATION

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	199	-	-
BETWEEN PHOSPHORUS LEVELS	4	1324	0.78
ERROR	195	1699	-

WAS USED IN THIS STUDY. THE PRIMARY DIFFERENCE BETWEEN THIS FEEDING TRIAL AND THAT OF THE PREVIOUS SECTION WAS THE TREATMENT OF THE DAMS FROM WHICH THE CHICKS WERE SECURED. THESE DAMS WERE FED THE NON-SUPPLEMENTED BREEDER RATION.

THE GAINS, FEED CONVERSIONS, AND KIDNEY ALKALINE PHOSPHATASE ACTIVITIES WERE OBTAINED FOR THE VARIOUS GROUPS AND THIS DATA IS PRESENTED IN TABLE LXX. AN ANALYSIS OF VARIANCE WAS MADE FROM THE DATA OBTAINED ON GAINS OF THE CHICKS FROM THIS TRIAL. THIS ANALYSIS IS PRESENTED IN TABLE LXXI. NO SIGNIFICANT DIFFERENCES WERE FOUND AMONG THE GAINS OF THE VARIOUS RATION TREATMENTS. THESE FINDINGS COMPARE FAVORABLY WITH THOSE OBTAINED IN THE CASE OF CHICKS, FROM TUNGSTATE SUPPLEMENTED DAMS, WHICH WERE FED THE SAME RATION AS REPORTED IN THE FIRST PORTION OF THIS STUDY.

A STATISTICAL ANALYSIS OF VARIANCE WAS THEN MADE ON THE FEED CONVERSION DATA OBTAINED BETWEEN THE VARIOUS GROUPS IN THESE TRIALS. THIS ANALYSIS IS SHOWN IN TABLE LXXII AND REVEALS THAT THERE WERE NO SIGNIFICANT DIFFERENCES AMONG THE VARIOUS TREATMENTS INVOLVED IN THIS STUDY.

THE ALKALINE PHOSPHATASE ACTIVITY REMAINED RELATIVELY CONSTANT IN ALL GROUPS.

TABLE LXXII

ANALYSIS OF VARIANCE OF FEED CONVERSIONS ON CHICKS, FROM NON-SUPPLEMENTED DAMS, FED PURIFIED DIET WITH PHOSPHORUS SUPPLEMENTATION

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	19	-	-
BETWEEN PHOSPHORUS LEVELS	4	23.50	1.18
ERROR	15	19.87	-

III. Practical Ration Studies With Vantress-White Plymouth Rock Chicks

In this trial commercial broiler chicks were fed a practical broiler ration supplemented with the various levels of phosphorus. Two hundred chicks were grown in 20 groups of 10 chicks each in this study. The gains, feed conversions, and alkaline phosphatase activities of kidney tissues obtained are presented in Table LXXIII.

An analysis of variance on the rates of gain is presented in Table LXXIV and shows that no significant differences in gain were attributable to the phosphorus supplementation.

TABLE LXXIII

Gain, Feed Conversions, and Alkaline Phosphatase Activity on Vantress-White Plymouth Rock Chicks Fed A Practical Ration With Phosphorus Supplementation

Added Phosphorus (ppm)	Gain (Grams)	Feed Conversion	Alkaline Phosphatase Activity*
0	305	1.69	115.6703
500	300	1.71	117.0781
1000	301	1.69	114.6519
1500	305	1.72	120.4065
2000	309	1.72	122.2550

*Alkaline phosphatase activity is expressed as milligrams phosphorus per gram kidney (wet weight).

THE ANALYSIS OF VARIANCE OF THE FEED CONVERSION DATA REVEALED THAT NO REAL DIFFERENCES EXISTED AMONG THE FEED CONVERSIONS DUE TO THE PHOSPHORUS SUPPLEMENTATIONS (TABLE LXXV).

THE ALKALINE PHOSPHATASE ACTIVITY LEVELS REMAINED RELATIVELY CONSTANT WHEN VARIOUS LEVELS OF PHOSPHORUS WERE ADDED TO THE RATION. THROUGHOUT THIS ENTIRE STUDY IT WAS NOTED THAT ADDITIONAL PHOSPHORUS DID NOT APPEAR TO HAVE AS MUCH EFFECT ON THE ALKALINE PHOSPHATASE ACTIVITY LEVELS AS WAS THE CASE WITH SULFATE SULFUR, COPPER, MOLYBDENUM, AND TUNGSTEN.

TABLE LXXIV

ANALYSIS OF VARIANCE ON GAIN FROM VANTRESS-WHITE PLYMOUTH ROCK CHICKS FED A PRACTICAL BROILER RATION WITH PHOSPHORUS SUPPLEMENTATION

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	199	-	-
BETWEEN PHOSPHORUS LEVELS	4	794	1.58
ERROR	195	504	-

TABLE LXXV

ANALYSIS OF VARIANCE ON FEED CONVERSIONS FROM VANTRESS-WHITE PLYMOUTH ROCK CHICKS FED A PRACTICAL BROILER RATION WITH PHOSPHORUS ADDITION

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	19	-	-
BETWEEN PHOSPHORUS LEVELS	4	82.50	0.87
ERROR	15	94.67	-

Summary

The additions of the various levels of phosphorus to the purified diet and the practical broiler ration resulted in no apparent effects on the gains, feed conversions, or alkaline phosphatase activities. Apparently the phosphorus content of the two basal rations was sufficient for optimum performance and the requirement for toxicity is high. Kidney alkaline phosphatase was not affected by the increased phosphorus intakes of these chicks.

It was concluded that phosphorus was not a limiting factor in the rations used in these studies with the other mineral elements.

H. Potassium Supplementation of Chick Diets

Since the additions of molybdenum, tungsten, and sulfur were in the form of sodium salts, it appeared desirable to study the effects of a potassium salt in these rations. Potassium was added as potassium chloride at levels of 0, 250, 500, 750, and 1000 ppm.

The basal rations used in this study were the same as used in the phosphorus study, that is, the purified ration plus 10 ppm molybdenum, one ppm tungsten, 30 ppm copper, and 1000 ppm sulfate sulfur. The practical broiler ration contained the same levels of supplementation except that copper was added at the level of 40 ppm.

The various trials involved in this study were of 20-day duration. Individual chick gain and feed conversions were obtained from each lot. Alkaline phosphatase activities of kidney tissues were determined on chicks from each group at the end of the experimental period.

1. Purified Ration Studies With Chicks From Tungsten Supplemented Dams

The chicks used in this portion of the study were from dams that had received a breeder ration supplemented with 500 ppm tungsten. This study involved one trial consisting of 200 chicks. The gains, feed conversions, and kidney alkaline phosphatase activities are presented in Table LXXVI.

An analysis of variance was made on the rate of gain of chicks in these trials and is presented in Table LXXVII. This revealed that potassium had no effect when added to the purified chick diet.

The statistical analysis of the feed conversions revealed that the addition of potassium to the purified diet was without effect. The results of this analysis is presented in Table LXXVIII.

TABLE LXXVI

RESULTS OF GAIN, FEED CONVERSIONS, AND ALKALINE PHOSPHATASE ACTIVITIES ON CHICKS, FROM TUNGSTATE DAMS, FED A PURIFIED RATION WITH VARIOUS LEVELS OF POTASSIUM

ADDED POTASSIUM (PPM)	GAIN (GRAMS)	FEED CONVERSION	ALKALINE PHOSPHATASE ACTIVITY*
0	259	1.49	54.7076
250	261	1.50	57.9824
500	264	1.50	60.8084
750	259	1.50	57.8343
1000	252	1.50	62.7739

* ALKALINE PHOSPHATASE IS EXPRESSED AS MILLIGRAMS PHOSPHORUS PER GRAM KIDNEY TISSUE (WET WEIGHT).

TABLE LXXVII

ANALYSIS OF VARIANCE ON GAIN OF CHICKS, FROM TUNGSTATE SUPPLEMENTED DAMS, FED A PURIFIED RATION WITH POTASSIUM SUPPLEMENTATION

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	199		
BETWEEN POTASSIUM LEVELS	4	794	1.58
ERROR	195	504	

TABLE LXXVIII

ANALYSIS OF VARIANCE ON FEED CONVERSIONS OF CHICKS, FROM TUNGSTATE SUPPLEMENTED DAMS, FED A PURIFIED RATION WITH POTASSIUM SUPPLEMENTATION

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	19		
BETWEEN POTASSIUM LEVELS	4	15	0.30
ERROR	15	50	

THE ALKALINE PHOSPHATASE VALUES WERE QUITE VARIABLE IN THIS TRIAL AND DID NOT DEMONSTRATE ANY CONSTANT TRENDS.

II. PURIFIED RATION STUDIES WITH CHICKS FROM NON-SUPPLEMENTED DAMS

THE CHICKS USED IN THIS TRIAL WERE FROM THE NON-SUPPLEMENTED WHITE PLYMOUTH ROCK DAMS. THE BASAL RATION USED WAS THE SAME AS REPORTED IN THE PREVIOUS SECTION. ONE TRIAL INVOLVING 200 CHICKS WAS CONDUCTED. THE GAINS, FEED CONVERSIONS, AND ALKALINE PHOSPHATASE DETERMINATIONS OBTAINED IN THIS STUDY ARE SHOWN IN TABLE LXXIX.

AN ANALYSIS OF VARIANCE WAS MADE ON THE RATE OF GAIN AND IS PRESENTED IN TABLE LXXX. THIS ANALYSIS SHOWED THAT THE ADDITON OF THE VARIOUS LEVELS OF POTASSIUM HAD NO EFFECT ON THE RATE OF GAINS. THIS IS IN AGREEMENT WITH THE FINDINGS OF THE STUDY REPORTED IN THE PREVIOUS SECTION.

AN ANALYSIS OF VARIANCE OF THE FEED CONVERSIONS IS SHOWN IN TABLE LXXXI. IT WAS REVEALED THAT THE ADDITION OF THE VARIOUS

TABLE LXXIX

GAIN, FEED CONVERSIONS, AND ALKALINE PHOSPHATASE ACTIVITIES OF CHICKS, FROM NON-SUPPLEMENTED DAMS, FED A PURIFIED DIET WITH POTASSIUM ADDITIONS

ADDED POTASSIUM (PPM)	GAIN (GRAMS)	FEED CONVERSION	ALKALINE PHOSPHATASE ACTIVITY*
0	260	1.49	66.5931
250	255	1.49	67.4057
500	263	1.46	66.6801
750	262	1.47	61.5489
1000	266	1.53	58.2739

* ALKALINE PHOSPHATASE ACTIVITY EXPRESSED IN MILLIGRAMS PHOSPHORUS PER GRAM KIDNEY TISSUE (WET WEIGHT)

TABLE LXXX

ANALYSIS OF VARIANCE ON GAIN OF CHICKS, FROM NON-SUPPLEMENTED DAMS, FED A PURIFIED DIET WITH POTASSIUM SUPPLEMENTATIONS

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	199		
BETWEEN POTASSIUM LEVELS	4	449	0.80
ERROR	195	567	

TABLE LXXXI

ANALYSIS OF VARIANCE ON FEED CONVERSIONS OF CHICKS, FROM NON-SUPPLEMENTED DAMS, FED PURIFIED DIET WITH POTASSIUM SUPPLEMENTATION

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	19	-	-
BETWEEN POTASSIUM LEVELS	4	36.75	1.85
ERROR	15	19.68	-

LEVELS OF POTASSIUM HAD NO BENEFICIAL EFFECTS ON THE FEED CONVERSION. THIS FINDING ALSO CONCURS WITH THOSE REPORTED IN THE PREVIOUS SECTION.

THE ALKALINE PHOSPHATASE ACTIVITIES FROM THESE CHICKS WERE INTERESTING. THE ADDITION OF 750 AND 1000 PPM POTASSIUM RESULTED IN CHICKS WHOSE KIDNEY ALKALINE PHOSPHATASE WAS LESS THAN THOSE OF THE BASAL RATION. THIS IS THE FIRST OBSERVATION OF THIS NATURE WITH ANY OF THE MINERAL ELEMENTS STUDIED.

III. Practical Ration Studies With Vantress-White Plymouth Rock Chicks

THE BASAL RATION USED IN THIS STUDY WAS THE CORN-SOYBEAN MEAL RATION. TWO SEPARATE TRIALS INVOLVING 100 CHICKS EACH WERE USED TO TEST THE EFFECT OF SUPPLEMENTAL POTASSIUM. THE GAIN, FEED CONVERSIONS, AND KIDNEY ALKALINE PHOSPHATASE ACTIVITIES WERE OBTAINED AND ARE PRESENTED IN TABLE LXXXII.

AN ANALYSIS OF VARIANCE OF THE RATE OF GAIN OF THE VARIOUS GROUPS INDICATED NO SIGNIFICANT DIFFERENCES AMONG THE LEVELS OF POTASSIUM FED IN THESE TRIALS. THE RESULTS OF THIS ANALYSIS IS PRESENTED IN TABLE LXXXIII.

TABLE LXXXII

RESULTS OBTAINED FROM VANTRESS-CROSS CHICKS FED PRACTICAL BROILER RATION WITH POTASSIUM SUPPLEMENTATION

ADDED POTASSIUM (PPM)	GAIN (GRAMS)	FEED CONVERSION	ALKALINE PHOSPHATASE ACTIVITY*
0	297	1.70	98.5787
250	298	1.74	103.5569
500	290	1.73	118.5796
750	296	1.69	110.9421
1000	290	1.73	119.8330

*ALKALINE PHOSPHATASE ACTIVITY IS EXPRESSED AS MILLIGRAMS PHOSPHORUS PER GRAM KIDNEY TISSUE (WET WEIGHT).

TABLE LXXXIII

ANALYSIS OF VARIANCE ON GAIN OF VANTRESS-CROSS CHICKS FED PRACTICAL RATION WITH VARIOUS POTASSIUM SUPPLEMENTATIONS

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	199	-	-
BETWEEN POTASSIUM LEVELS	4	376.2	0.44
ERROR	195	864.0	-

TABLE LXXXIV

ANALYSIS OF VARIANCE ON FEED CONVERSIONS OF VANTRESS-CROSS CHICKS FED PRACTICAL BROILER RATION WITH POTASSIUM SUPPLEMENTATION

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE
TOTAL	19	-	-
BETWEEN POTASSIUM LEVELS	4	15.80	0.46
ERROR	15	34.27	-

A STATISTICAL ANALYSIS MADE ON THE FEED CONVERSION DATA (TABLE LXXXIV) REVEALED NO REAL DIFFERENCES IN FEED CONVERSIONS AMONG ANY OF THE GROUPS IN THESE TRIALS.

THE ALKALINE PHOSPHATASE ACTIVITIES AGAIN WERE A POINT OF INTEREST. THERE WERE NO CONSISTANT TRENDS APPARENT WHEN THESE VALUES WERE COMPARED TO THE LEVELS OF POTASSIUM SUPPLEMENTATION. THIS WAS SOMEWHAT SURPRISING SINCE THE CHICKS FROM THE HENS FED THE NON-SUPPLEMENTED BREEDER RATION TENDED TO HAVE LOWER KIDNEY ALKALINE PHOSPHATASE ACTIVITIES WHEN FED THE 750 AND 1000 PPM LEVELS OF POTASSIUM.

SUMMARY

THE SUPPLEMENTATION OF THE BASAL RATIONS WITH POTASSIUM DID NOT INFLUENCE THE GAIN OR FEED CONVERSION. THE KIDNEY ALKALINE PHOSPHATASE VALUES WERE SOMEWHAT VARIABLE AND DID NOT FOLLOW ANY CONSISTANT TRENDS. THE ACTIVITIES OF THE CHICKS, FROM UNSUPPLEMENTED BREEDER HENS, WERE LOWER IN THE CASE OF 750 AND 1000 PPM OF POTASSIUM ADDITIONS. THIS IS THE FIRST TIME IN ANY OF THE STUDIES IN WHICH THE INCREASED MINERAL ADDITIONS DECREASED THE ALKALINE PHOSPHATASE VALUES.

THIS DECREASE IN ENZYME VALUES WAS NOT OBSERVED IN CHICKS, FROM TUNGSTATE SUPPLEMENTED DAMS, OR THE COMMERCIAL BROILERS FED THE PRACTICAL RATION. IT WOULD APPEAR THAT POTASSIUM WAS NOT A LIMITING FACTOR IN RATIONS USED IN THESE STUDIES SINCE THERE WAS NO EFFECT ON GAINS, FEED CONVERSIONS, OR ALKALINE PHOSPHATASE ACTIVITIES.

SUMMARY

THE RESULTS OF THREE-WEEK CHICK GROWTH TRIALS INVOLVING SEVERAL MINERAL ELEMENTS ARE REPORTED. SINCE TUNGSTEN HAD BEEN REPORTED TO BE A METABOLIC ANTAGONIST TO MOLYBDENUM AND SINCE MOLYBDENUM WAS TO BE THOROUGHLY INVESTIGATED, TUNGSTEN WAS FED IN THE RATION OF BREEDER HENS FOR SOME OF THE CHICKS USED IN THIS STUDY. WHITE PLYMOUTH ROCK CHICKS, FROM TUNGSTATE-SUPPLEMENTED AND NON-SUPPLEMENTED DAMS, WERE FED A BASAL PURIFIED DIET AND VANTRESS-WHITE PLYMOUTH ROCK CROSSBRED CHICKS WERE FED A BASAL PRACTICAL CORN-SOYBEAN MEAL RATION. THESE CHICKS AND BASAL RATIONS WERE USED TO TEST THE EFFECTS ON GROWTH, FEED CONVERSIONS, AND CERTAIN ENZYME LEVELS, OF SIX MINERAL ELEMENTS. THE ELEMENTS INVOLVED IN THESE STUDIES WERE MOLYBDENUM, TUNGSTEN, COPPER, SULFUR, PHOSPHORUS, AND POTASSIUM.

SHORT TERM EXPERIMENTAL STUDIES (24, 48, AND 72 HOURS) WERE MADE WITH DEUTECTOMIZED CHICKS AND CHICKS ON WHICH THERE HAD BEEN NO OPERATION, IN WHICH AD LIBITUM AND FORCED FEEDING WAS USED. IT WAS FOUND THAT THE DEUTECTOMIZED CHICKS GREW AT A SIGNIFICANTLY SLOWER RATE THAN THE CONTROL CHICKS REGARDLESS OF THE METHOD OF FEEDING. IT WAS FELT THAT THIS TYPE CHICK EXPERIMENTS WERE NOT ADEQUATE FOR MOLYBDENUM ASSAY WORK.

THE ADDITION OF 500 PPM TUNGSTEN TO THE DIET OF BREEDER HENS RESULTED IN A DECREASED XANTHINE DEHYDROGENASE ACTIVITY IN THE LIVER, KIDNEY, AND INTESTINAL TISSUES OF THE HENS ALTHOUGH NO EFFECT WAS

OBSERVED UPON THE ADDITION OF 250 PPM TUNGSTEN. TUNGSTEN AT EITHER LEVEL DID NOT APPEAR TO HAVE AN EFFECT ON THE RATE OF EGG PRODUCTION OR THE PER CENT HATCHABILITY OF THE EGGS FROM THESE HENS. DAY-OLD CHICKS FROM THESE SUPPLEMENTED DAMS WERE FOUND TO HAVE MORE XANTHINE DEHYDROGENASE IN LIVER AND INTESTINAL TISSUES THAN DID CHICKS FROM THE NON-TUNGSTATE SUPPLEMENTED DAMS. THIS DOES NOT AGREE WITH WORK REPORTED BY HIGGINS AND ASSOCIATES (1956) IN WORK WITH RATS. IT WAS REPORTED THAT TUNGSTEN ADDITIONS LOWERED THE XANTHINE OXIDASE ACTIVITY IN LIVER TISSUES. IT SHOULD BE POINTED OUT THAT THERE EXISTS THE POSSIBILITY OF A DIFFERENCE IN XANTHINE OXIDASE IN RATS AND XANTHINE DEHYDROGENASE IN CHICKS. DUE TO THE LARGE AMOUNT OF VARIATION NO SIGNIFICANT DIFFERENCES WERE FOUND IN XANTHINE DEHYDROGENASE AT THE END OF A FOUR-WEEK GROWTH PERIOD EVEN WHEN THE CHICKS WERE FED A TUNGSTATE-SUPPLEMENTED RATION.

THERE WAS A CARRY-OVER OF TUNGSTEN FROM DAMS TO CHICKS, AS THE ELEMENT WAS FOUND IN HIGH CONCENTRATIONS IN KIDNEY TISSUES OF CHICKS FROM TUNGSTATE-SUPPLEMENTED DAMS. THIS CONDITION RESULTED IN SIGNIFICANT GROWTH DEPRESSIONS FOR AT LEAST FOUR WEEKS. THIS CONDITION WAS NOT ONLY OVERCOME BUT SIGNIFICANT GAIN INCREASES WERE OBTAINED, COMPARED TO CHICKS FROM NON-SUPPLEMENTED DAMS, WHEN MOLYBDENUM WAS ADDED TO THE RATIONS. THIS WAS FOUND BOTH IN PURIFIED AND PRACTICAL RATION STUDIES, WHICH SUGGESTED A SYNERGISTIC EFFECT BETWEEN MOLYBDENUM AND TUNGSTEN IN THE RATIONS OF GROWING CHICKS.

BEST RESULTS FROM MOLYBDENUM, ALONE OR IN COMBINATION WITH TUNGSTEN, WERE RECORDED WHEN ADDED AT THE LEVEL OF 10 PPM. THIS IS AT CONSIDERABLY HIGHER LEVELS THAN THOSE REPORTED (KURNICK ET AL 1957, REID ET AL 1956A, 1956B, 1957) IN WHICH MAXIMAL RESPONSES WERE OBTAINED AT LEVELS OF LESS THAN ONE PPM. THE LEVEL AT WHICH MOLYBDENUM CAUSED DEPRESSIONS IN GAIN AND FEED CONVERSION VARIED FROM 500 PPM IN CHICKS

FROM TUNGSTATE SUPPLEMENTED DAMS TO 1000 PPM IN THE DIETS OF CHICKS FROM NON-SUPPLEMENTED DAMS.

TUNGSTEN, WHEN ADDED AT LEVELS OF ONE OR 10 PPM TO THE RATIONS, HAD NO BENEFICIAL EFFECTS WHEN ADDED ALONE, BUT WHEN THERE WAS A RATIO OF MOLYBDENUM:TUNGSTEN OF 1:1 OR 10:1, SIGNIFICANT INCREASES IN GAIN AND FEED CONVERSION WERE OBSERVED EVEN IN CHICKS IN WHICH THE SINGLE ADDITION OF MOLYBDENUM HAD RESULTED IN AN INCREASE IN GAIN.

IN RATIONS CONTAINING SUPPLEMENTAL LEVELS OF 10 PPM MOLYBDENUM AND ONE PPM TUNGSTEN, COPPER WAS FOUND TO SIGNIFICANTLY IMPROVE THE RATIONS. BEST RESULTS WERE OBTAINED WHEN COPPER WAS ADDED AT 30 PPM AND 40 PPM FOR THE PURIFIED DIET AND THE PRACTICAL BROILER RATION, RESPECTIVELY. TOTAL COPPER CONTENTS WERE 55.9 (PURIFIED DIET) AND 50.8 IN THE CASE OF THE PRACTICAL BROILER RATION. THE SUPPLEMENTAL COPPER CAUSED INCREASES IN GAIN REGARDLESS OF THE DAM TREATMENT, ALTHOUGH GREATER GAINS WERE FOUND IN THE CASE OF CHICKS FROM TUNGSTATE FED DAMS. THIS FACT SUGGESTED THAT COPPER MIGHT HAVE OVERCOME A TUNGSTATE TOXICITY IN THESE CHICKS DUE TO THE CARRYOVER OF TUNGSTEN FROM THE BREEDER HENS. ANOTHER POSSIBILITY EXISTS AND THIS IS THAT COPPER MAY BE ACTING SYNERGISTICALLY WITH EITHER MOLYBDENUM OR TUNGSTEN.

THE ADDITION OF 1000 AND 2000 PPM INORGANIC SULFUR TO A PURIFIED DIET TO WHICH HAD BEEN ADDED 10 PPM MOLYBDENUM, ONE PPM TUNGSTEN, AND 30 PPM COPPER RESULTED IN A SIGNIFICANT INCREASE IN RATES OF GAIN FOR CHICKS REGARDLESS OF WHETHER THE DAMS RECIEVED TUNGSTATE SUPPLEMENTATIONS. THE ADDITION OF 1000 PPM INORGANIC SULFUR TO THE PRACTICAL RATION RESULTED IN A SIGNIFICANT INCREASE IN GAIN. THIS WAS OF INTEREST AS THE RATIONS ALREADY CONTAINED 11,649 AND 4,635 PPM SULFUR FOR THE PURIFIED AND THE PRACTICAL RATION, RESPECTIVELY. EVIDENCE

FROM THIS STUDY SUPPORTS THE PROPOSAL (GORDON ET AL 1954, MACHLIN 1955) THAT CHICKS MAY HAVE A REQUIREMENT FOR INORGANIC SULFATE PER SE.

NO BENEFICIAL EFFECTS WERE FOUND FROM THE ADDITION OF PHOSPHORUS OR POTASSIUM TO THE RATIONS USED IN THIS STUDY. BOTH PHOSPHORUS AND POTASSIUM WERE TESTED IN THE PURIFIED RATION (CONTAINING SUPPLEMENTAL ADDITIONS OF 10 PPM MOLYBDENUM, ONE PPM TUNGSTEN, 30 PPM COPPER, AND 1000 PPM INORGANIC SULFUR) AND IN THE PRACTICAL BROILER RATION WHICH CONTAINED THE SAME SUPPLEMENTAL LEVELS OF THE ELEMENTS AS ABOVE EXCEPT THAT COPPER WAS ADDED AT 40 PPM. PHOSPHORUS OR POTASSIUM DID NOT APPEAR TO BE LIMITING FACTORS IN THE RATIONS USED IN THESE STUDIES.

URIC ACID SYNTHESIS WAS DETERMINED FROM LIVER AND KIDNEY TISSUE HOMOGENATES IN TWO STUDIES. THE RATE OF URIC ACID SYNTHESIS DID NOT APPEAR TO BE CORRELATED TO ANY OF THE CRITERIA OBTAINED IN EITHER OF THE STUDIES OR TO THE LEVELS OF MOLYBDENUM OR TUNGSTEN SUPPLEMENTATIONS. URIC ACID SYNTHESIS WAS STUDIED AS AN ALTERNATE METHOD OF STUDYING XANTHINE OXIDATION.

ALKALINE PHOSPHATASE ACTIVITIES WERE OF CONSIDERABLE INTEREST IN EACH OF THE STUDIES. AS A RULE THE ENZYME ACTIVITIES INCREASED SLIGHTLY WITH INCREASING LEVELS OF MINERAL SUPPLEMENTATIONS. WHEN THE SUPPOSED "TOXIC LEVELS" WERE REACHED, GROWTH WAS DEPRESSED AND WAS ACCOMPANIED BY A DRAMATIC RISE IN ALKALINE PHOSPHATASE ACTIVITY. EXCEPTIONS WERE NOTED UPON ADDITIONS OF SULFUR AND COPPER TO CHICKS HATCHED FROM TUNGSTEN SUPPLEMENTED DAMS AND FED THE PURIFIED DIET WHERE GROWTH DEPRESSIONS DID NOT OCCUR. IT IS BELIEVED THAT THERE IS A DIRECT OR INDIRECT RELATIONSHIP BETWEEN METABOLIC STRESS(ES) AND THE ALKALINE PHOSPHATASE ACTIVITY, AND WHEN THIS CONDITION IS BROUGHT ABOUT

IT IS REFLECTED BY A RISE IN THE ENZYME ACTIVITY. IT IS THOUGHT THAT THE HIGH MINERAL SUPPLEMENTATIONS PRODUCED SUCH A STRESS CONDITION WHICH WAS REFLECTED BY THE DRAMATIC RISE IN THE ALKALINE PHOSPHATASE ACTIVITY.

CONCLUSIONS

From the data presented in this study the following conclusions are warranted.

(1) There is a carry-over of tungsten from the dam to the chick and one of the primary sites of storage for this element is the kidney.

(2) There is a depression in the rate of gain for a period of at least four weeks in these chicks, presumably due to the high tungsten stores.

(3) Molybdenum appears to be effective not only in overcoming this tungstate effect but these elements appear to be synergistic in chick rations, especially in certain ratios.

(4) Copper was effective in causing increases in chick gains when added in rations containing molybdenum and tungsten, regardless of whether the dams received supplemental tungsten in the diet.

(5) The inclusion of inorganic sulfate in rations containing molybdenum, tungsten, and copper resulted in significant stimulation of chick gain. This supports the proposal that there is a chick requirement for inorganic sulfate <u>per se</u>.

(6) Supplemental molybdenum, tungsten, or molybdenum plus tungsten appeared to have no effect on the rate of uric acid synthesis.

(7) ALKALINE PHOSPHATASE ACTIVITY APPEARS TO INCREASE DRAMATICALLY DURING PERIODS OF METABOLIC STRESS.

(8) ELEMENTS, SUCH AS THE ONES DISCUSSED, SHOULD BE STUDIED COLLECTIVELY RATHER THAN INDIVIDUALLY. THERE APPEAR TO BE DEFINITE RELATIONSHIPS OR INTERRELATIONSHIPS BETWEEN THE MINERAL ELEMENTS REPORTED IN THIS STUDY.

(9) DELETERIOUS EFFECTS ON RATE OF GROWTH AT HIGH LEVELS OF SUPPLEMENTATION OF MOLYBDENUM, TUNGSTEN, AND COPPER WERE OBSERVED IN THIS STUDY. THIS WAS ACCOMPANIED BY HIGH ALKALINE PHOSPHATASE ACTIVITY.

APPENDIX I

A METHOD FOR THE DETERMINATION OF TRACE QUANTITIES OF MOLYBDENUM AND TUNGSTEN IN FEEDS, FEED INGREDIENTS, AND ANIMAL TISSUES

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IN AN ATTEMPT TO STUDY THE INTAKE OF THE SO CALLED "TRACE ELEMENTS", A RAPID METHOD FOR THEIR DETERMINATION WAS SOUGHT. SINCE THE CONTENT OF MOLYBDENUM AND TUNGSTEN IS VERY LOW IN POULTRY RATIONS, NORMALLY ONLY A FEW PARTS PER MILLION, CONSIDERABLE DIFFICULTY WAS ENCOUNTERED WHEN METHODS PRESENTLY IN THE LITERATURE WERE USED.

USING MODIFICATION OF SEVERAL METHODS (1, 2) AND SEVERAL DIFFERENT TECHNIQUES, IT IS FELT THAT THE METHOD DESCRIBED BELOW IS FAIRLY RAPID AND HAS BEEN SHOWN TO GIVE EXCELLENT RESULTS.

APPARATUS AND REAGENTS

ALL ABSORBANCE MEASUREMENTS WERE MADE IN 1.00 CM. CELLS WITH A BECKMAN DU SPECTROPHOTOMETER.

NITRIC ACID, CONCENTRATED, A.R.

PERCHLORIC ACID, 60 - 70%

40% AMMONIUM CITRATE: PREPARE BY USING LEAD-FREE CITRIC ACID PLUS CONCENTRATED AMMONIUM HYDROXIDE TO pH 8.5 AND EXTRACTING WITH DITHIZONE. (USED TO PREVENT PRECIPITATION WHEN SOLUTION IS MADE ALKALINE).

DITHIOL:* DISSOLVE 1.0 GRAM OF DITHIOL IN 200 ML OF N-AMYL ACETATE

* 4-METHYL-1,2-DIMERCAPTOBENZENE, COMMONLY CALLED DITHIOL, PURCHASED FROM EASTERN CHEMICAL CORP, NEWARK, N. J.

(WHICH CONTAINS 0.5% THIOGLYCOLLIC ACID, TO PREVENT RAPID LOSS IN COLOR IN THE TUNGSTEN DETERMINATION). PREPARE FRESH DAILY. STORE IN REFRIGERATOR UNDER AN INERT ATMOSPHERE OF NITROGEN.

2 N AMMONIUM HYDROXIDE

STANNOUS CHLORIDE. DISSOLVE 20 GMS OF STANNOUS CHLORIDE DIHYDRATE IN 50 ML OF HYDROCHLORIC ACID AND DILUTE TO 100 ML WITH WATER. IT MAY BE NECESSARY TO HEAT, MODERATELY, TO OBTAIN CLEAR SOLUTION.

TITANIUM SPONGE, OBTAINED FROM ELECTRO METALLURGICAL CO., NIAGARA FALLS, NEW YORK.

DITHIZONE REAGENT: DISSOLVE 0.5 GM OF COMMERCIAL DITHIZONE (DIPHENYL-THIOCARBAZONE) IN ABOUT 500 ML OF CARBON TETRACHLORIDE. FILTER INTO A FIVE-LITER SEPARATORY FUNNEL CONTAINING 2 TO 3 LITERS OF 0.02 N AMMONIUM HYDROXIDE. SHAKE WELL, EXTRACTING THE DITHIZONE INTO THE AQUEOUS PHASE. SEPARATE THE CCl_4 AND USE AGAIN TO DISSOLVE THE REMAINING DITHIZONE ON THE FILTER PAPER. FILTER AGAIN INTO THE SAME SEPARATORY FUNNEL. REPEAT THIS PROCESS UNTIL LITTLE OR NO RESIDUE REMAINS ON THE FILTER PAPER. AFTER THE LAST EXTRACTION DISCARD THE CCl_4 PHASE. EXTRACT THE AMMONICAL SOLUTION OF DITHIZONE WITH FRESH 50 ML. PORTIONS OF CCl_4 UNTIL TRACES OF PINK NO LONGER APPEAR IN THE CCl_4 LAYER. ADD ABOUT 500 ML. CCl_4 AND ACIDIFY WITH HCL WITH SHAKING UNTIL THE DITHIZONE IS EXTRACTED FROM THE AQUEOUS PHASE INTO THE CCl_4 LAYER. DILUTE TO 1 LITER WITH CCl_4. STORE IN A BROWN GLASS STOPPERED BOTTLE IN A COLD DARK PLACE. THE REAGENT SHOULD REMAIN STABLE FOR SEVERAL WEEKS.

PROCEDURE:

WEIGH A SAMPLE OF FINELY GROUND MIXED FEED OR FEED INGREDIENTS OF SUCH SIZE AS TO CONTAIN ABOUT 40 MICROGRAMS OF MOLYBDENUM AND/OR TUNGSTEN. IN THE CASE OF ANIMAL TISSUE, IT NEED NOT BE GROUND, AS WHOLE CARCASSES OF YOUNG CHICKS WERE USED INTACT. TRANSFER TO A 600-ML TALL-FORM PYREX BEAKER AND ADD SUFFICIENT HNO_3 TO DISSOLVE THE SAMPLE. LET STAND UNTIL FOAMING CEASES. IN MOST CASES IT WAS FOUND DESIRABLE TO LET STAND OVER-NIGHT. ATTENTION SHOULD BE CALLED TO THE FACT THAT SOME FEEDS FOAM EXCESSIVELY SHORTLY AFTER THE ADDITION OF HNO_3 AND MUST BE STIRRED RAPIDLY TO PREVENT LOSS FROM FOAMING OVER. ADD 2 OR 3 PIECES OF VAPOGLAS OR GLASS BEADS AND PLACE ON A HOT PLATE AT LOW HEAT. CONTINUE ADDING NITRIC ACID, AS NEEDED, UNTIL THE MAJOR

PORTION OF THE ORGANIC MATTER HAS BEEN DESTROYED (SAMPLE REMAINS CLEAR EVEN WHEN THE VOLUME IS REDUCED TO ALMOST DRYNESS). WHEN SAMPLE CONTAINS CONSIDERABLE FAT MUCH MORE HNO_3 IS NEEDED FOR DESTRUCTION. REMOVE BEAKER FROM HOT PLATE AND ADD 25-ML. HNO_3 AND 5-ML. $HClO_4$. CONTINUE DIGESTION ON LOW HEAT, THEN MEDIUM HEAT UNTIL SAMPLE IS JUST DRY, BUT FREE OF $HClO_4$ FUMES. COOL.

ADD 5-ML. HCL, HEAT GENTLY TO DISSOLVE ASH AND TRANSFER QUANTITATIVELY TO A 250-ML. SEPARATORY FUNNEL, DILUTE TO APPROXIMATELY 50-ML. WITH DISTILLED WATER. ADDED 10-ML. 40% AMMONIUM CITRATE SOLUTION (pH 8.5), AND ADJUST pH OF SOLUTION TO 8.5 WITH 7 N NH_4OH, USING 10-12 DROPS OF M-CRESOL PURPLE AS AN INDICATOR. ADD AN EXCESS OF CONCENTRATED DITHIZONE REAGENT (50-60 ML.) AS INDICATED BY A RUSTY-ORANGE AQUEOUS PHASE AFTER SHAKING (TWO OR MORE ADDITIONS OF DITHIZONE MAY BE REQUIRED FOR THIS). DRAIN THE CCL_4 PHASE AND EXTRACT AQUEOUS PHASE WITH SUCCESSIVE 25-ML. PORTIONS OF CCL_4 UNTIL THE CCL_4 LAYER IS CLEAR GREEN.

WASH THE AQUEOUS LAYER INTO A 600-ML PYREX TALL-FORM BEAKER WITH WATER, ADD GLASS BEADS AND 25-ML. HNO_3 AND EVAPORATE ON A HOT PLATE UNTIL VOLUME IS REDUCED TO ABOUT 15-ML. ADD 15-ML. HNO_3 AND 5-ML. $HClO_4$ AND DIGEST TO DRYNESS. COOL AND ADD 2-ML. CONCENTRATED H_2SO_4 AND HEAT TO FUMES. COOL.

USING A TOTAL VOLUME OF 15-ML. OF HCL (1 TO 2), CAREFULLY PUT SAMPLE IN SOLUTION (THIS MAY REQUIRE MODERATE HEAT) AND TRANSFER TO A 250-ML. ERLENMEYER FLASK. IF NECESSARY, SAMPLE MAY BE TRANSFERRED AND EXCESS VOLUME REDUCED BY EVAPORATING ON A HOT PLATE.

MOLYBDENUM DETERMINATION:

COOL SAMPLE TO 20° C. ADD 3-ML. STANNOUS CHLORIDE SOLUTION AND

10-ML. OF DITHIOL SOLUTION. SHAKE FOR TEN MINUTES. TRANSFER TO A SEPARATORY FUNNEL USING 3 TO 5-ML. WASHINGS OF N-AMYL ACETATE (WHICH CONTAINS 0.5% THIOGLYCOLLIC ACID). KEEP ORGANIC LAYER TO LESS THAN 20-ML. WHEN LAYERS HAVE SEPARATED, DRAW OFF ACID LAYER INTO ORIGINAL FLASK AND RESERVE FOR SUBSEQUENT TUNGSTEN DETERMINATION. WASH THE AMYL ACETATE LAYER WITH TWO 2 TO 3 - ML. PORTIONS OF HCL (1 TO 2) AND RETURN THESE WASHINGS TO THE FLASK.

THE ACETATE LAYER NOW CONTAINS THE MOLYBDENUM. THE MOLYBDENUM CAN BE DETERMINED AT THIS POINT BY DILUTING TO 25-ML. WITH AMYL ACETATE AND MEASURING THE ABSORBENCE AT 685MU. PREPARE A REAGENT BLANK BY CARRYING THROUGH ALL THE STEPS OF THE PROCEDURE AND USE AS THE REFERENCE SOLUTION IN THE SPECTROPHOTOMETER. OBTAIN THE AMOUNT OF MOLYBDENUM BY REFERRING TO A STANDARD CURVE PREPARED BY MEASURING THE ABSORBENCE OF SOLUTIONS CONTAINING KNOWN AMOUNTS OF STANDARD SODIUM MOLYBDATE SOLUTIONS RANGING FROM 20 TO 200 MCG. MOLYBDENUM/25-ML. AMYL ACETATE.

TUNGSTEN DETERMINATION:

ADD 25-ML. HCL AND 0.2 GM. TITANIUM SPONGE TO THE RESERVED SOLUTION (ACID LAYER IN MOLYBDENUM DETERMINATION) AND HEAT GENTLY UNTIL THE SOLUTION HAS TURNED TO A MEDIUM PURPLE DUE TO THE PRESENCE OF TITANIUM (III). ADD 10-ML. DITHIOL SOLUTION AND HEAT 20 MINUTES IN A WATER BATH AT 85°C, SWIRLING THE FLASK FREQUENTLY.

TRANSFER THE SOLUTION TO A SEPARATORY FUNNEL USING SMALL WASHINGS OF AMYL ACETATE (WITH ADDED THIOGLYCOLLIC ACID) AND KEEPING THE VOLUME OF THE ORGANIC LAYER TO LESS THAN 20-ML. DRAW OFF AND DISCARD THE ACID LAYER. WASH THE ACETATE LAYER TWICE WITH 3 TO 5-ML. HCL (4 TO 1). TRANSFER THE AMYL ACETATE LAYER TO A DRY 25-ML. VOLUMETRIC FLASK,

DILUTE TO VOLUME AND MIX. MEASURE THE ABSORBENCE IMMEDIATELY AT 640MU, USING AMYL ACETATE AS A REFERENCE SOLUTION. OBTAIN THE TUNGSTEN BY REFERRING TO A STAND CURVE PREPARED FROM KNOWN AMOUNTS OF SODIUM TUNGSTATE STANDARD SOLUTIONS RANGING FROM 20 TO 200 MCG. TUNGSTEN/ 25-ML. AMYL ACETATE.

COMMENTS:

IN CASES WHERE THE KNOWN MOLYBDENUM AND TUNGSTEN CONTENT OF SAMPLES IS LOW (LESS THAN 10 MCG. IN SAMPLE USED), IT MAY BE NECESSARY TO ADD SUFFICIENT STANDARD SOLUTIONS TO ADJUST EITHER OR BOTH ELEMENTS TO THE PROPER LEVELS. PRELIMINARY SAMPLES SHOULD BE RUN TO DETERMINE THE LEVELS OF MOLYBDENUM AND TUNGSTEN AND RECOVERIES MUST BE CHECKED FOR BOTH. WHEN SAMPLE SIZE IS LARGE (25-50 GMS.), OTHER ELEMENTS MAY BE PRESENT IN SUFFICIENT QUANTITIES TO INTERFERE WITH THE RESULTS. AN ALKALINE EXTRACTION WITH DITHIZONE WILL REMOVE COPPER (WHICH IN OUR LABORATORY HAS BEEN SHOWN TO INTERFERE), COBALT, LEAD, AND ZINC WHILE LEAVING THE MOLYBDENUM AND TUNGSTEN FREE. THE REMOVAL OF INTERFERING IONS BY THIS MEANS IS FAIRLY RAPID AND INSURES A RELIABLE DETERMINATION.

SULFURIC ACID IN LARGE QUANTITIES INTERFERES WITH THE FINAL DETERMINATIONS AND CANNOT BE USED IN THE WET-ASHING PROCEDURE OF THE ORIGINAL SAMPLES. CARE MUST BE TAKEN DURING THE SECOND WET ASHING (TO DESTROY THE EXCESS DITHIZONE AND CITRATE) TO ADD JUST ENOUGH ACID TO AID IN THE REMOVAL OF THE LAST TRACES OF PERCHLORIC ACID.

IF IT IS DESIRABLE TO OMIT THE DETERMINATION OF MOLYBDENUM, ADD THE STANNOUS CHLORIDE SOLUTION AND PROCEED WITH THE <u>TUNGSTEN DETERMINATION</u> PROCEDURE. THE PRESENCE OF MOLYBDENUM WILL NOT INTERFERE.

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APPENDIX II

URIC ACID DETERMINATIONS FROM LIVER AND KIDNEY

APPARATUS AND REAGENTS:

(1) ALL ABSORBENCE MEASUREMENTS WERE MADE IN 1.0 CM CELLS WITH A BECKMAN DU SPECTROPHOTOMETER.

(2) POTTER - ELVEHJEM TYPE HOMOGENIZER.

(3) DUBNOFF METABOLIC SHAKING INCUBATOR.

(4) STANDARD URIC ACID SOLUTION - WEIGH OUT ON A WATCH GLASS EXACTLY 1 GRAM OF URIC ACID AND TRANSFER IT TO A LITER VOLUMETRIC FLASK BY MEANS OF A NOT TOO SMALL, DRY FUNNEL. TAP THE FUNNEL, SO AS TO TRANSFER NEARLY THE WHOLE OF THE URIC ACID TO THE FLASK. TRANSFER 0.6 GRAM OF LITHIUM CARBONATE TO A 250 MILLILITER FLORENCE FLASK, ADD 150 MILLILITERS OF WATER; SHAKE ABOUT FIVE MINUTES UNTIL DISSOLVED. SOME INSOLUBLE MATERIAL REMAINS, AND IT IS USUALLY BEST TO FILTER. HEAT THE SOLUTION OR FILTRATE TO 60° C. ALSO, WARM THE LITER FLASK UNDER RUNNING WARM WATER. POUR THE WARM LITHIUM CARBONATE SOLUTION INTO THE LITER FLASK, INCIDENTALLY WASHING INTO IT THE TRACES OF URIC ACID WHICH ADHERED TO THE WATCH GLASS AND FUNNEL. SHAKE SO AS TO DISSOLVE THE URIC ACID PROMPTLY. A LITTLE ADDITIONAL WARMING UNDER HOT TAP WATER IS PERMISSIBLE. THE LITHIUM CARBONATE SOLUTION IS NOT ALWAYS PERFECTLY CLEAR EVEN WHEN FILTERED, AND ONE SHOULD NOT MISTAKE THIS LITTLE TURBIDITY FOR UNDISSOLVED URIC ACID AND KEEP WARMING AND SHAKING TOO LONG. IN FIVE MINUTES ALL OF THE URIC ACID SHOULD BE DISSOLVED. SHAKE THE FLASK UNDER COLD RUNNING WATER WITHOUT UNDUE DELAY. ADD 20 MILLILITERS OF 40 PER CENT FORMALIN, AND HALF FILL THE FLASK WITH DISTILLED WATER. FINALLY ADD, FROM A PIPETTE RATHER SLOWLY AND WITH SHAKING, 25 MILLILITERS OF NORMAL SULFURIC ACID. DILUTE TO VOLUME, MIX THOROUGHLY, AND TRANSFER TO A CLEAN, TIGHTLY STOPPERED BOTTLE. THIS STOCK SOLUTION, CONTAINING 1 MILLIGRAM OF URIC ACID PER MILLILITER, SHOULD BE KEPT AWAY FROM LIGHT.

TO PREPARE THE WORKING STANDARD, DILUTE 1 MILLILITER OF THE STOCK SOLUTION, WITH WATER ONLY, TO 250 MILLILITERS. IT BEHAVES EXACTLY LIKE A LITHIUM CARBONATE SOLUTION OF URIC ACID AND KEEPS PERFECTLY FOR MANY DAYS. (5 ML. = 0.02 MG. URIC ACID.)

(5) Urea - Fifty grams urea dissolved in sufficient water to make 100 cc. The solution keeps well at room temperature.

(6) Arsenophosphotungstic acid - One hundred gms. of pure sodium tungstate are placed in a liter pyrex flask and dissolved in about 600 milliliters of water. Then 50 grams of pure arsenic acid (As_2O_5) followed by 25 milliliters of 85 per cent phosphoric acid and 20 milliliters of concentrated hydrochloric acid are added. The mixture is boiled for about 20 minutes, cooled and diluted to 1 liter. It is stable indefinately.

(7) Sodium cyanide, 12 per cent.

(8) Xanthine, 0.05 molar - Dissolve 0.0761 grams xanthine in 10 milliliters of 0.05 normal sodium hydroxide.

(9) Methylene Blue, 0.0113 Molar - Dissolve 0.4215 gram methylene blue in 100 milliliters water.

(10) Michaelis Universal Buffer, pH 7.42 - Add 9.714 grams of sodium acetate (containing 3 molecules of water of hydration) and 14.714 grams of the sodium salt of veronal (barbital) to sufficient water to dissolve it and make up to 500 milliliters. To each 5 milliliters of this solution is added 2 milliliters of an 8.5 per cent sodium chloride solution plus 5 milliliters of 0.1 normal hydrochloric acid and 13 milliliters of water. This buffer is isotonic with blood.

(11) Trichloroacetic acid, 30 per cent.

PROCEDURE:

The tissue was homogenized with an appropriate amount of Michaelis' buffer, pH 7.42, to give a 1:10 dilution of the tissue. A 2 milliliter aliquot of the homogenate was taken and added to a 25 milliliter Erlenmeyer flask containing 4 milliliters of the buffer to which had been added 0.15 milliliter of 0.0113 molar methylene blud. Three flasks were treated in the above. The flasks were then incubated for 10 minutes at 40°C in the Dubnoff metabolic shaking incubator. At the end of this period 1 milliliter of 30 per cent trichloroacetic acid was added to one of the flasks and it was removed from the Dubnoff. Into a second flask 0.1 milliliter of .05 molar xanthine was added.

At the end of an additional 20 minute incubation period, 1 milliliter of 30 per cent trichloroacetic acid was added to the remaining two flasks. The contents were shaken to insure complete mixing, and the contents of each of the three flasks were transferred to 15 milliliter centrifuge tubes and centrifuged for 15 minutes. A 2 milliliter aliquot of the supernatent fluid was then taken (the aliquot from the flask to which the xanthine had been added was diluted 1:6 with water and a 2 milliliters aliquot of this dilution was taken) and transferred to a test tube graduated at 25 milliliters. To each tube was then added 2 milliliters portions of the following: urea solution, sodium cyanide solution and arseno-phosphotungstic acid. The tubes were then diluted to 25 milliliters, mixed thoroughly and allowed to stand 50 minutes for the color to develop. A blank tube was prepared along with the incubated flasks, without the tissue homogenate.

The amount of uric acid synthesized was determined by subtracting the "10 + 20 minute" endogenous uric acid from that amount obtained from the flask which contained the added xanthine.

All readings were made at 890 mu, since it was found that methylene blue does not interfere at this wavelength, and there is a peak in the absorbence of the uric acid at this point.

APPENDIX III

DETERMINATION OF ALKALINE PHOSPHATASE

APPARATUS AND REAGENTS:

(1) ALL ABSORBENCE MEASUREMENTS WERE MADE IN 1.0 CM. CELLS WITH A BECHMAN D. U. SPECTROPHOTOMETER.

(2) POTTER - ELVEHJEM TYPE HOMOGENIZER.

(3) DUBNOFF METABOLIC SHAKING INCUBATOR.

(4) MICHAELIS' UNIVERSAL BUFFER pH 7.42. - DISSOLVE 9.714 GRAMS OF SODIUM ACETATE (CONTAINING 3 MOLECULES OF WATER OF HYDRATION) AND 14.714 GRAMS OF THE SODIUM SALT OF VERONAL (BARBITAL) IN WATER AND MADE UP TO 500 MILLILITERS. TO EACH 5 MILLILITERS OF THIS SOLUTION IS ADDED 2 MILLILITERS OF AN 8.5 PER CENT SODIUM CHLORIDE SOLUTION PLUS 5 MILLILITERS OF 0.1 NORMAL HYDROCHLORIC ACID AND 13 MILLILITERS OF WATER. THIS BUFFER IS ISOTONIC WITH BLOOD.

(5) MOLYBDATE II - DISSOLVE 25 GRAMS OF REAGENT-GRADE AMMONIUM MOLYBDATE IN ABOUT 200 MILLILITERS OF WATER. IN A 1-LITER VOLUMETRIC FLASK PLACE 300 MILLILITERS OF 10 NORMAL SULFURIC ACID. ADD THE MOLYBDATE SOLUTION AND DILUTE WITH WASHINGS TO 1 LITER WITH WATER AND MIX. IT IS STABLE INDEFINITELY.

(6) AMINONAPTHOLSULFONIC ACID REAGENT - PLACE 195 MILLILITERS OF 15 PER CENT SODIUM BISULFITE SOLUTION IN A GLASS STOPPERED CYLINDER. ADD 0.5 GRAM OF 1, 2, 4-AMINONAPTHOLSULFONIC ACID, THEN ADD 5 MILLILITERS OF 20 PER CENT SODIUM SULFITE. STOPPER AND SHAKE UNTIL POWDER IS DISSOLVED.

(7) ALKALINE PHOSPHATE SUBSTRATE - INTO A 100 MILLILITERS VOLUMETRIC FLASK INTRODUCE SUCCESSIVELY 3 MILLILITERS OF PETROLEUM ETHER, ABOUT 80 MILLILITERS OF DISTILLED WATER, 0.5 GRAM OF SODIUM B-GLYCEROPHOSPHATE, 0.424 GRAM OF SODIUM DIETHYL BARBITURATE, AND WATER TO VOLUME (READ AT INTERFACE BETWEEN PATROLEUM ETHER AND AQUEOUS SOLUTION). KEEP IN THE REFRIGERATOR.

(8) TRICHLORACETIC ACID - 30 PER CENT AND 5 PER CENT.
STANDARD PHOSPHATE SOLUTION - DISSOLVE EXACTLY 0.351 GRAM OF PURE DRY MONOPOTASSIUM PHOSPHATE IN WATER AND TRANSFER QUANTITATIVELY TO A 1 LITER VOLUMETRIC FLASK. ADD 10 MILLILITERS OF 10 NORMAL SULFURIC ACID, DILUTE TO THE MARK WITH

WATER, AND MIX. THIS SOLUTION CONTAINS 0.4 MILLIGRAM PHOSPHORUS IN 5 MILLILITERS. IT IS STABLE INDEFINITELY. FROM THE ABOVE SOLUTION, PLACE 6.25 MILLILITERS IN A 100 MILLILITERS VOLUMETRIC FLASK. ADD 16.7 MILLILITERS OF 30 PER CENT TRICHLORACETIC ACID SOLUTION, DILUTE TO 100 MILLILITERS WITH WATER AND MIX. THIS SOLUTION CONTAINS 0.04 MILLIGRAM OF PHOSPHORUS IN 8 MILLILITERS IN 5 PER CENT TRICHLORACETIC ACID.

PROCEDURE:

THE TISSUE WAS HOMOGENIZED WITH AN APPROPRIATE AMOUNT OF MICHAELIS BUFFER, pH 7.42, TO GIVE A 1:10 DILUTION OF THE ORIGINAL TISSUE. A 1 MILLILITER ALIQUOT OF THE HOMOGENATE WAS THEN ADDED TO 9 MILLILITERS OF "ALKALINE PHOSPHATE" SUBSTRATE WHICH HAD BEEN MEASURED INTO TWO 25 MILLILITER ERLENMEYER FLASKS EQUIPPED WITH RUBBER STOPPERS. THIS WAS ALLOWED TO INCUBATE IN THE DUBNOFF METABOLIC SHAKER FOR 15 MINUTES AT 40° C. THE FLASKS WERE THEN REMOVED, COOLED IN ICE WATER FOR SEVERAL MINUTES AND 2 MILLILITERS OF 30 PER CENT TRICHLORACETIC ACID WAS ADDED. THE SOLUTIONS WERE MIXED, TRANSFERRED TO CENTRIFUGE TUBES AND PLACED IN A CENTRIFUGE. BLANK VALUES WERE OBTAINED FROM WHICH SAMPLES WERE TREATED IN THE SAME MANNER EXCEPT THAY WERE NOT INCUBATED. AFTER CENTRIFUGING A 2 MILLILITER ALIQUOT OF THE SUPERNATENT WAS TAKEN AND ADDED TO A TEST TUBE GRADUATED AT 12.5 MILLILITERS. TO EACH TUBE WAS THEN ADDED 1 MILLILITER OF MOLYBDATE II REAGENT AND THE CONTENTS THOROUGHLY MIXED. THEN 0.4 MILLILITER OF AMINONAPTHOL-SULFONIC ACID REAGENT WAS ADDED TO EACH TUBE, DILUTED TO 12.5 MILLI-LITERS WITH WATER AND THE CONTENTS THOROUGHLY MIXED. THEY WERE ALLOWED TO STAND FOR 5 MINUTES FOR COLOR DEVELOPMENT. A BLANK WAS PREPARED BY USING 8 MILLILITERS OF 5 PER CENT TRICHLOROACETIC ACID AND ADDITIONS OF MOLYBDATE II AND ASMINOSULFONIC ACID REAGENT.

THE PHOSPHATASE ACTIVITY WAS CALCULATED AS THE DIFFERENCE BETWEEN THE INORGANIC PHOSPHATE CONTENT OF THE INCUBATED AND UNINCUBATED SAMPLES AND EXPRESSED AS MILLIGRAMS OF PHOSPHORUS PER GRAM OF ANIMAL TISSUE (WET WEIGHT BASIS). ALL PHOTOMETER READINGS WERE MADE AT 660 MU.

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AUTOBIOGRAPHY

I was born March 3, 1930, the son of James Harvey and Ada Smith Teekell, of Hineston, Louisiana. I attended elementary school in that area and was graduated from Oak Hill High School of Elmer, Louisiana, in May, 1947. I entered Northwestern State College, Natchitoches, Louisiana, in September, 1947, and transferred to Louisiana State University in September, 1949. I received a Bachelor of Science degree from Louisiana State University with a major in Vocational Agricultural Education in June 1951, after having finished my course work in January of that year.

On February 3, 1951, I enlisted in the United States Air Force, and after six months was transferred to Germany where I served for two years and four months, until I was released from active duty on December 17, 1953.

On January 31, 1953, I was married to Helena Mildred Delaney, of Nowata Oklahoma. We have a son, James Delaney, born July 24, 1954, and a daughter, Gwen Marie, born September 20, 1956.

In February, 1954, I was admitted to the Graduate School of Louisiana State University. I received a Master of Science Degree with a major in Poultry Husbandry in August, 1955.

I then entered a program leading to a Doctor of Philosophy degree with a major in Poultry Nutrition and a minor in Biological Chemistry.

EXAMINATION AND THESIS REPORT

Candidate: ROGER ALTON TEEKELL

Major Field: POULTRY NUTRITION AND BIOCHEMISTRY

Title of Thesis: THE EFFECT OF MOLYBDENUM, TUNGSTEN, COPPER, INORGANIC SULFUR, PHOSPHORUS AND POTASSIUM IN AVIAN NUTRITION

Approved:

A B Watts
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JULY 17, 1958